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Exobiology and Future Mars Missions

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Exobiology and Future Mars Missions

Edited by
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Moffett Field, California

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EXOBIOLOGY AND FUTURE MARS MISSIONS

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Supported by NASA's Exobiology Program Office
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The editors wish to thank Ron Teeter for his help in the preparation of this report.

CONFERENCE SUMMARY

The objectives of this workshop were to consider the scientific questions associated with exobiology on Mars and to determine how these questions could be addressed on future Mars missions. The mission that provided a focus for discussions was the Mars Rover/Sample Return Mission—which is currently in the planning stages. The international nature of future Mars missions was underscored by the participation in the workshop of two Soviet space scientists.

During the meeting Dr. Lev Mukhin, from the Moscow Institute for Space Sciences, stated that the USSR is in a position to launch missions to Mars today but needs US scientific input to achieve exobiology science objectives. He invited scientific participation in the Soviet missions, an invitation that was well received by the conference attendees.

Dr. Dale Compton, the director (acting) of NASA Ames Research Center opened the conference and welcomed the participants. His comments reaffirmed the Center's commitment to play a leadership role in exobiology and in future Mars explorations.

The conference sessions covered 5 main themes:

- Review of current plans for Mars Rover Sample Return Mission.
- Continuing the search for life on Mars.
- The biogenic elements and chemical evolution on Mars.
- Searching for evidence of past life on Mars.
- The Antarctic and other analogs—the importance of field work.

In addition there was a brief presentation of plans for future human exploration missions and the role humans might play in exobiology. It was clear from this discussion that this topic was of considerable interest and needed to be discussed in more detail. Another topic that was mentioned but not discussed in detail was the issue of planetary protection, or quarantine. There are plans to have followup workshops on both of these topics.

The overall conclusion of the conference was that the scientific priorities for exobiology were (in order):

1. Understanding the environmental conditions on the present Mars and during the early period of Martian history when conditions may have been more suitable for life.
2. Search for fossil evidence of past life on Mars. A lot can be learned from studies of Earth's earliest biosphere to guide the search for fossils of a possible early Martian biota. Fossil traces might include organic material, stromatolites, etc.
3. While it is unlikely that there is life on Mars today, the discovery of life would be of enormous interest. Therefore the possibility of continuing the search for life on Mars should be considered, albeit as a reduced scientific priority.

It was clear from the many presentations that there is a wealth of knowledge and experience, mostly gained from field studies of life or fossil remains of life on Earth, that can be brought to bear on the design and implementation of exobiology experiments on a Mars Rover/Sample Return mission.

BRIEF SUMMARIES OF THE MAIN SESSIONS

Presented here are brief summaries of each of the main themes considered in the conference.

1. Review of current plans for Mars Rover Sample Return Mission. The Mars Sample Return Mission is currently under study. The range of missions under consideration span the possibilities from a limited-range, possibly tethered rover, to a highly capable long-range semi-autonomous rover. The range of the long term rover is on the order of 10–100 km. The sample return mass is not yet well constrained but a working figure of 5 kg is being used by mission designers. The current science objectives of this mission include:

> **"Assess the molecular, isotopic and morphologic evidence for prebiotic evolution and the possible origin of life during the history of Mars."**

Current plans for this mission would involve a new start in 1992 and a launch in 1998. The sample would be returned near the beginning of the next century.

2. Continuing the search for life on Mars. The virtually unanimous consensus among the scientist at the meeting was that it is highly improbable that there is life present on Mars. Despite rumors that the Soviet Mars program placed a high priority on continuing the search for extant life; Lev Mukhin and Mikhail Marov, both agreed that extant life is unlikely. The overall conclusion seems to be to accord searches for extant life to a lower scientific priority while acknowledging the importance of the discovery of extant life on Mars.

3. The biogenic elements and chemical evolution on Mars. The standard hypothesis of the origin of life is based upon the chemical evolution of organic material. Evidence from the outer solar system, comets, and the interstellar medium suggests that abiotic reactions forming complex organic material are common. To understand the environment on Mars and its potential or past potential for life we must understand the biogenic elements and chemical evolution on the planet. The Viking results are the first step in this process. Viking detected no organic compounds in the soils tested. This appears to be due to the presence of an oxidant in the Martian soil. However the presence of water appears to remove the oxidant. Thus there is considerable interest in determining sites of current water activity on Mars and looking there for organic material. In addition, it is possible that organic material may be sequestered in sedimentary deposits laid down during the early periods of Martian history under more clement conditions. Calculations indicate that organic material on Mars could conceivably have survived several billions of years under Mars conditions.

4. Searching for evidence of past life on Mars. There is considerable geological (fluvial features, sedimentary deposits,) and geochemical evidence (N-isotopes) that Mars' climate was much different in the past, and more conducive to biology. Recent models suggest that the duration of this early clement period was comparable to the time required for the origin of life on Earth. If Earth and Mars were indeed similar in this early epoch it is plausible to suppose that life arose on Mars. Since Mars has greatly reduced erosion and tectonic activity compared to Earth the record of these early events may be more readily found on Mars. Thus, although Mars may not have life on it today it may hold the best record of the events that lead to life. Looking for fossil evidence of this early life on Mars is a possible goal for Martian exploration. To determine how to approach this task, the session participants considered how evidence for the earliest life on Earth has been gleaned from the fossil record. The environments on early Earth and the adaptive strategies organisms developed to survive and grow in these environments; including hot springs, playal lakes, lakeshores, etc. can be used as a guide for what to look for on Mars and where.

5. The Antarctic and other analogs—the importance of field work. Looking for life on Mars or ev-

idence of past life will require a certain knowledge of life and the adaptive strategies that life takes in cold and dry conditions. For this reason the study of cold and dry environments on Earth are relevant to plans for future exobiological studies of Mars. The most well studied analog environment is the dry valleys of Antarctica. In addition studies of organisms that live on or near snow and ice, desert microorganisms, and the survival of dehydrated organisms are giving new insights into life at low water activity.

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EARTH'S EARLY FOSSIL RECORD:
WHY NOT LOOK FOR SIMILAR FOSSILS ON MARS?

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There is general consensus that the early geological history of Mars was similar to that of Earth. If chemical evolution occurred on Mars and life evolved, did evolution follow a pathway similar to that postulated for Earth: heterotrophs appearing first, followed sometime later by autotrophs? Was solar radiation a sufficiently important resource to have favored the development of photoautotrophy? And, even if solar radiation was important, was enough time available for the evolution of this remarkable microbial physiology whose microbial remains and sedimentary constructions appear to have the best chance of being preserved?

The search for the most ancient fossils on Earth has centered on slightly metamorphosed sedimentary rocks in Early Archean terrains. Stromatolites and microfossils, two conventional types of fossils, are actively sought, but so are rocks with potential chemical fossil evidence (kerogen and biologically fractionated isotopes).

To date, the oldest evidence of life on Earth consists of stromatolites, microbial fossils and kerogen that have been found in approximately 3500 Ma-old rocks in Western Australia and South Africa. Carbon of the kerogen is isotopically light with δ^{13}C values between -26.6 to $-32.0\,^{\circ}/_{\circ\circ}$ for Swaziland (South African) and -31.2 to $-34.3\,^{\circ}/_{\circ\circ}$ for Warrawoona (Australian) cherts, suggesting autotrophic activity. Eight morphotypes of microbial fossils (six filamentous and two coccoidal) have been detected in bedded chert from these strata. In terms of their size and shape, these microbial fossils resemble a variety of modern prokaryotes and thus make precise taxonomic assignment very difficult if not impossible. The original chemical composition of the fossils has been so severely altered that biochemical taxonomic procedures standard in microbiology are useless. Nevertheless, the morphology and organization of some of the Warrawoona fossils, e.g., the larger (>3 μm in diameter) tubular and septated filaments and the two pluricellular coccoidal microfossils, are sufficiently similar to some modern cyanobacteria that a taxonomic affiliation with this group seems reasonable. The occurrence of these microbial fossils in stromatolite-like, wavy to irregularly laminated (laminae are 5 to 500 μm thick) chert suggests a microbial mat habit for the organisms and supports the cyanobacterial comparison. However, the overall appearance of the bedded chert in hand specimen does not closely resemble a stratiform stromatolite.

As spectacular as these microbial fossils may be (the preservation of bacteria in rock has always fascinated geologists and biologists), the most impressive and paleobiologically significant fossils are the macroscopic stromatolites found at a few localities in both regions. Stromatolites are organosedimentary structures produced by the sediment trapping, binding and/or precipitation activity of benthic microbes, principally photoautotrophs and usually cyanobacteria. The dynamic interaction of microbes and sediment can produce laminated sedimentary structures that range in geometry from domes and columns centimeters to meters in diameter, to wavy laminated stratiform sediments millimeters to meters in thickness. Stromatolites are found throughout the geological column and are found forming today in a wide variety of environments. The Early Archean stromatolites consist of centimeter-sized domes, pseudocolumns and stratiform constructions which morphologically resemble many younger examples. No microbial fossils have been detected in these stromatolites but have been found in laminated chert at localities several kilometers away.

Based on our biogeological understanding of stromatolite formation in the past and present, Early Archean stromatolites indicate the following: (1) early in their history, prokaryotes developed an episedimentary to epilithic habit in shallow aqueous environments, some of which were periodically exposed; (2) the microbes actively influenced sediment accumulation at their habitat site; (3) the microbes possessed tropic and/or taxic responsive behavior to the stimulus of sunlight that kept them at or near the sediment-fluid interface; (4) stromatolite-building microbes were probably fast-growing and/or motile (to keep up with sedimentation); (5) the constructing microbes had some minimum resistance to high-energy solar radiation although their sedimentary context and sheaths may have reduced this factor; and (6) communities of several different taxa participated in the construction. The diversity of morphologically complex microbes and the presence of stromatolites in deposits 3500 Ma old indicate that life evolved rapidly on early Earth. If indeed cyanobacteria had evolved by this time (the circumstantial evidence permits such an assumption), this suggests that most, if not all, of the major prokaryotic metabolic pathways had evolved by 3500 Ma ago (if the cyanobacteria were oxygen releasing, then even aerobes could have evolved).

Preservation of the pre-Phanerozoic microbial record is a capricious and selective process. In order for these earliest stromatolites to be fossilized, mineral matter must be precipitated to cement the accumulation of biologically accreted or produced material. This must occur early in the accretion of the stromatolite or early during diagenesis, before the stromatolite undergoes compaction which would alter diagnostic characteristics of its micro- and macrostructure. Calcium carbonate is the most common cementing agent, primarily because the microbes that build stromatolites are photoautotrophs utilizing carbon dioxide and can influence the precipitation of calcium carbonate. At times, chert replaces the carbonate of the stromatolite. Chert can also be a primary chemical sediment. The fossilization of microbes is a much more specialized and variable process. Since microbes do not possess skeletons, it is important that after death, they do not undergo much abiological and biological decomposition. Otherwise, changes can result in shrinkage, decrease in structural integrity and loss of important morphological information. Permineralization of microbes by silica in the form of chert is the best geological phenomenon known for long term preservation (hundreds of millions to billions of years). Calcium carbonate, another geologically readily available cementing and embedding medium, does not form a sufficiently impermeable medium for long term microbial preservation. This is one of the main reasons why stromatolites, which are primarily composed of carbonates, do not normally contain preserved microbes.

If the lessons learned from the study of Earth's earliest fossil record are to be applied to Mars, certain sedimentary rock types and sedimentary rock configurations should be targeted for investigation and returned by the Martian rover and ultimately by human explorers. 1. Domical, columnar to wavy laminated stratiform sedimentary rocks that resemble stromatolites should be actively sought. Limestone, other carbonates and chert are the favored lithology. Being macroscopic, stromatolites might be recognized by an intelligent unmanned rover. 2. Black, waxy chert with conchoidal fracture should be sought. Chert is by far the preferred lithology for the preservation of microbes and chemical fossils. One lesson we have learned from studies of ancient life on Earth is that even under optimal geological conditions (little or no metamorphism, little or no tectonic alteration, excellent outcrops, good black chert) and with experienced field biogeologists, the chances of finding well preserved microbial remains in chert are very low. Serendipity appears to play a major role.

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VIKING BIOLOGY EXPERIMENTS AND THE MARTIAN SOIL

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The Viking Biology Experiments (VBE) are the most informative database on the wet chemistry and reactivity of the Martian soil available today. The simulation and chemical interpretation of their results have given us valuable hints towards the characterization of the soils' mineralogy, adsorption properties, pH and redox. The characterization of Mars soil on the basis of ten years of LR and other VBE simulations will be reviewed.

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MICROBIAL MATS IN PLAYA LAKES AND OTHER SALINE HABITATS: EARLY MARS ANALOG?

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Microbial mats are cohesive benthic microbial communities which inhabit various Terra (Earth-based) environments including the marine littoral and both permanent and ephemeral (playa) saline lakes.[1] The Terran fossil record for such communities (stromatolites and microfossils) extends back to ca $\geq 3.5 \times 10^9$ years before present, a time when it is considered probable that Mars possessed a warmer, more humid climate than it has at present.[2] Certain geomorphological features of Mars, such as the Margaritifer Sinus, have been interpreted as ancient, dried playa lakes, presumably formed before or during the transition to the present Mars climate. Studies of modern Terran examples suggest that microbial mats on early Mars would have had the capacity to survive and propagate under environmental constraints that would have included irregularly fluctuating regimes of water activity and high ultraviolet flux. Assuming that such microbial communities did indeed inhabit early Mars, their detection during the MRSR mission depends upon the presence of features diagnostic of the prior existence of these communities or their component microbes or, as an aid to choosing suitable landing, local exploration or sampling sites, geomorphological, sedimentological or chemical features characteristic of their playa lake habitats. Examination of modern Terran playas (e.g., the Lake Eyre basin) shows that these features span several orders of magnitude in size (Fig. 1). For example, ephemeral stream floodplains and eolian dune fields are large-scale features consistent with playa lake systems. Smaller-scale features include those indicative of groundwater emergence (e.g., mound springs, megapolygons), subaerial exposure (e.g., desiccation polygons), or hypersaline conditions (e.g., evaporite minerals such as gypsum and halite). While stromatolites are commonly centimeter–meter scale features, bioherms or "fields" of individuals may extend to larger scales. Desiccation, though preventing bacterial degradation of non-lithified mats, produces structures vulnerable to physical destruction/removal via eolian erosion.[3] Preservation of organic matter (mats and microbes) would, however, be favored in topographic lows such as channels or ponds of high salinity particularly those receiving silica-rich groundwaters. These areas are likely to be located near former zones of groundwater emergence and/or where flood channels entered the paleo-playa. Fossil playa systems which may aid in assessing the applicability of this particular Mars analog include the Cambrian Observatory Hill Beds of the Officer Basin[4] and the Eocene Wilkins Peak Member of the Green River Formation.[5]

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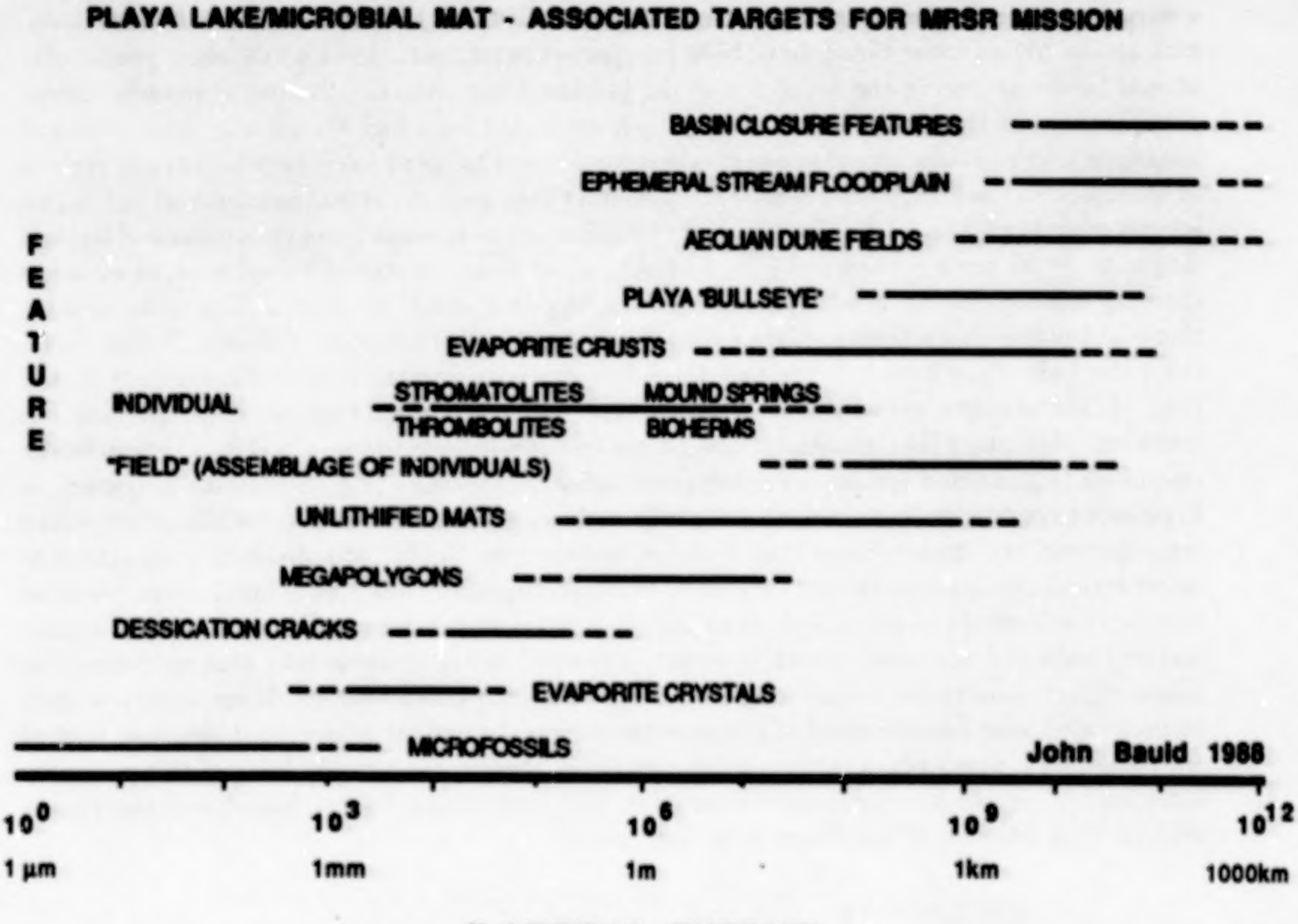

Figure 1. Approximate dimensions of biogenic and environmental targets diagnostic of, or consistent with, the former presence of saline/playa lake-hosted microbial mat communities on Mars.

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STABLE ISOTOPE LASER SPECTROSCOPY

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Recent advances in semiconductor laser technology have produced a reliable lightweight device ideally suited for a spacecraft high resolution molecular spectrometer. Lead-salt tunable diode lasers (TDL) emit in several spectral modes, each with a very narrow linewidth of $\sim 0.0003\ \mathrm{cm}^{-1}$. This spectral resolution is much narrower than typical Doppler broadened molecular linewidths in the mid-IR range. Thus, it is possible to detect individual rotational lines within the vibrational band and measure their intensity, which can be used to determine gas concentration. Moreover, at such high spectral resolution, problems of impurity gases interfering with the measurement can be eliminated. The narrow spectral lines of any impurity gas tend to lie between the narrow lines of the gas of interest. This represents a major advantage over the accepted gas chromatograph mass spectrometer (GCMS) technique for measuring gas concentrations and isotope ratios. The careful and extensive gas purification procedures required to remove impurities for reliable GCMS measurements will not be required for an IR laser gas analysis.

We are developing the infra-red laser gas analysis technique to measure stable isotopic ratios of gases such as CO_2, CH_4, N_2O and NH_3. This will eventually lead to development of instruments capable of *in situ* isotopic measurements on planets such as Mars. The carbon ($^{12}C/^{13}C$) isotope ratio is indicative of the type of carbon fixation mechanisms (e.g., photosynthesis, respiration) in operation on a planet, while the nitrogen ($^{14}N/^{15}N$) isotope ratio can probably be used to date nitrogen-bearing Martian samples.

We have recently measured the absorbance ratio of two adjacent lines of CO_2 in the $2300\ \mathrm{cm}^{-1}$ (4.3 micron) region of the spectrum. The precision of the measurement is presently better than 1% and significant improvement is anticipated as we incorporate rapid sweep-integration techniques and computer controlled data acquisition capabilities.

In addition to application to a Mars Rover, this technique has potential use in many areas of interest to NASA, such as determination of pressure, temperature, and chemical reaction rates in a shock wave, monitoring the environment of a space station, and field equipment for ground truth studies of atmosphere-biosphere interactions.

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ANALYTICAL ELECTRON MICROSCOPY OF BIOGENIC AND INORGANIC CARBONATES

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In the terrestrial sedimentary environment, the mineralogically predominant carbonates are calcite-type minerals (rhombohedral carbonates) and aragonite-type minerals (orthorhombic carbonates). Most common minerals precipitating either inorganically or biogenically are high magnesium calcite and aragonite. High magnesium calcite (with magnesium carbonate substituting for more than 7 mole % of the calcium carbonate) is stable only at temperatures greater than 700 °C or thereabouts, and aragonite is stable only at pressures exceeding several kilobars of confining pressure. Therefore, these carbonates are expected to undergo chemical stabilization in the diagenetic environment to ultimately form stable calcite and dolomite.

Because of the strong organic control of carbonate deposition in organisms during biomineralization, the microchemistry and microstructure of invertebrate skeletal material is much different than that present in inorganic carbonate cements. The style of preservation of microstructural features in skeletal material is therefore often quite distinctive when compared to that of inorganic carbonate even though wholesale recrystallization of the sediment has taken place.

In this brief presentation, microstructural and microchemical comparisons will be made between high magnesium calcite echinoderm skeletal material and modern inorganic high magnesium calcite inorganic cements, using Analytical Electron Microscopy and related techniques. Similar comparisons will be made between analogous materials which have undergone stabilization in the diagenetic environment.

Similar analysis schemes may prove useful in distinguishing between biogenic and inorganic carbonates in returned Martian carbonate samples.

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Rationale For A Mars Rover/Sample Return Mission

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A Mars Rover/Sample Return mission is currently being studied for the late 1990's. The objectives of the mission are to better understand the origin and evolution of Mars, to search for evidence of former life, and to improve our knowledge of the Martian environment in preparation for subsequent human exploration. Among the planets, Mars has long been of special interest because of the possibility that life might have started there, and because of the certainty that it will be the first planet to be visited by humans. Having formed in a different part of the Solar System from Earth, Mars will provide clues that will better enable us to discriminate between conflicting theories of Solar System formation. Mars is also a natural laboratory on which a wide range of geologic and meteorological processes have operated under conditions very different from those on Earth. Samples are needed so that the full range of analytical techniques available here on Earth can be applied to the study of these issues. The rover provides the mobility needed to access different materials, and can be equipped with an analytical capability so that the planet can be sampled intelligently. The rover will also provide the means of exploring the planet on a human scale and performing a wide range of *in situ* measurements at different locations. Different mission scenarios are currently being studied with the goal of achieving sample return before the end of the century.

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USE OF NEAR INFRARED CORRELATION SPECTROSCOPY FOR QUANTITATION OF SURFACE IRON, ABSORBED WATER AND STORED ELECTRONIC ENERGY IN A SUITE OF MARS SOIL ANALOG MATERIALS

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A number of questions concerning the surface mineralogy and the history of water on Mars remain unresolved using the Viking analyses and Earth-based telescopic data. Identification and quantitation of iron-bearing clays on Mars would elucidate these outstanding issues. Near infrared correlation analysis, a method typically applied to qualitative and quantitative analysis of individual constituents of multicomponent mixtures, is adapted here to selection of distinctive features of a small, highly homologous series of Fe/Ca-exchanged montmorillonites and several kaolinites. Independently determined measures of surface iron, relative humidity and stored electronic energy were used as "constituent" data for linear regression of the constituent vs. reflectance data throughout the spectral region 0.68–2.5 μm. High correlations were found in appropriate regions for all three constituents, though that with stored energy is still considered tenuous. Quantitation was improved using 1st and 2nd derivative spectra. High resolution data over a broad spectral range would be required to quantitatively identify iron-bearing clays by remotely sensed reflectance.

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LIFE WITHOUT WATER

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Anhydrobiosis, or "life without water" is commonly demonstrated by a number of plants and animals. Organisms that can become anhydrobiotic have been known and studied since Leewuenhoek first described them in 1702. These organisms have the capacity to lose all their body water, remain dry for various periods, and then be revived by rehydration. They can be divided into two distinct groups, those organisms which become anhydrobiotic at some early developmental stage such as fungal or bacterial spores, seeds, chironomid larvae, or encysted gastrulae of the brine shrimp *Artemia salina*; and those organisms which can be dried out as adults, such as soil or moss-dwelling rotifers, nematodes, and tardigrades. The latter category is useful for studying characteristics of anhydrobiotic organisms and the mechanisms of survival since the anhydrobiotic state is not complicated by developmental processes.

While in the anhydrobiotic state, these organisms become highly resistant to several environmental stresses such as extremely low temperatures (0.05 K), elevated temperatures (around 100° C), ionizing radiation, and high vacuum (10^{-3} to 10^{-9} Torr). Their survival is increased by storage in the dark and the absence of 0_2, suggesting that adventitious chemistry may play a role in their eventual failure to revive after long periods of storage.

The question of whether anhydrobiotic organisms show metabolism has not been directly answered. One experiment has shown a very low level of 0_2 uptake at a relative humidity of 25%. However, these organisms can survive temperatures as low as 0.05 K, at which temperature metabolic processes must be at a virtual standstill. Calculations on the hydration of proteins show that at the hydration levels commonly experienced by anhydrobiotic organisms, all the water is immobile and there is about one water molecule/polar group of the protein. At such hydrations, it is difficult to conceive how enzymatic processes might proceed. Thus, anhydrobiotic organisms most likely have suspended metabolism.

Since water is commonly thought to be essential for life, a major question is: How do anhydrobiotic organisms survive the almost total loss of water? Our laboratory has shown that during the slow drying essential to survival, nematodes manufacture large quantities of the disaccharide trehalose. More recently, other laboratories have shown that the ability of yeast to survive drying is strongly correlated with trehalose content, and that during repeated cycles of hydration and drying bacterial spore resistance to drying decreases as the trehalose content decreases. A search of the literature reveals that many anhydrobiotic organisms make large quantities of trehalose or other carbohydrates.

Further experiments in our laboratory have shown that trehalose is able to stabilize and preserve dry microsomes of sarcoplasmic reticulum and artificial liposomes. With a number of physical studies we have demonstrated that trehalose and other disaccharides can interact directly with phospholipid headgroups and maintain membranes in their native configuration by replacing water in the headgroup region. Our most recent studies show that trehalose is an effective stabilizer of proteins during drying and that it does so by direct interaction with groups on the protein.

If life that is able to withstand environmental extremes has ever developed on Mars, one would expect such life to have developed some protective compounds which can stabilize macromolecular structure in the absence of water and at cold temperatures. On Earth, that role appears to be filled by carbohydrates that can stabilise both membrane and protein structures during freezing and drying. By analogy with terrestrial systems, such life forms might develop resistance either during some reproductive stage or at any time during their adult existence.

If the resistant form is a developmental stage, the life cycle of the organism must be completed within a reasonable time period relative to time when environmental conditions are favorable (on Mars or Earth) so that a new resistant developmental stage can be produced. This would suggest that simple organisms with a short life cycle might be most successful.

Anhydrobiotic organisms (either at some developmental stage or as adults) rapidly metabolize excess trehalose when they are rehydrated. Thus, adult organisms that undergo repeated cycles of rehydration and drying must spend sufficient time in the rehydrated and dehydrating periods to re-synthesize trehalose stores in order to survive another drying cycle.

Other environmental factors that favor prolonged survival in the anhydrobiotic state are the absence of oxygen and light.

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STABLE CARBON AND SULFUR ISOTOPES AS RECORDS OF THE EARLY BIOSPHERE

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The abundance ratios of the stable isotopes of light elements such as carbon and sulfur can differ between various naturally-occurring chemical compounds. If coexisting compounds have achieved mutual chemical and isotopic equilibrium, then the relative isotopic compositions of these compounds can record the conditions (e.g., temperature) at which equilibrium was last maintained. If coexisting chemical compounds indeed formed simultaneously but had not achieved mutual equilibrium, then their relative isotopic compositions often reflect the conditions and mechanisms associated with the kinetically-controlled reactions responsible for their production. In the context of Mars, the stable isotopic compositions of various minerals (and organic compounds?) might record not only the earlier environmental conditions of that planet, but also whether or not the chemistry of life ever occurred there.

Two major geochemical reservoirs occur in Earth's crust, both for carbon (carbonates and reduced organic carbon) and sulfur (sulfates and sulfides). In rocks formed in low-temperature sedimentary environments, the oxidized forms of these elements (the carbonates and sulfates) tend to be enriched in the isotope having larger mass (^{13}C, ^{34}S), relative to the reduced forms (organics and sulfides). In sediments where the organics and sulfides were formed by biological processes, these isotopic contrasts have been caused by the processes of biological CO_2 fixation and dissimilatory sulfate reduction. Because these isotopic patterns are pervasive in sedimentary rocks, and, in addition, they persist through geologic time despite frequent thermal alteration of their host rocks, these patterns have become important indicators of the antiquity of life on Earth.

Such isotopic contrasts between oxidized and reduced forms of carbon and sulfur are permitted by thermodynamics at ambient temperatures. Thus they do not necessarily require the intervention of life. However, nonbiological chemical reactions associated with the production of organic matter and the reduction of sulfate are extremely slow at ambient temperatures. Thus the ubiquitous synthesis of organics and sulfides under ambient conditions illustrates life's profound role as a chemical catalyst that has altered the chemistry of Earth's crust.

For carbon, the isotopic "signature" of biological CO_2 fixation is the remarkably consistent two to four percent depletion in the $^{13}C/^{12}C$ value of organic matter, relative to carbonate, in sedimentary rocks. This isotopic contrast is, in most cases, due to the activity of the enzyme ribulose bisphosphate carboxylase, which incorporates the CO_2 into a sugar molecule. The persistence of this isotopic contrast in rocks ranging from the Recent to more than 3.5 billion years ago might very well testify to the extreme age of this key enzyme.

For sulfur, the isotopic signature of bacterial sulfate reduction is the presence in sediments of sulfide which has a wide range of $^{34}S/^{32}S$ values that are generally lower than the corresponding values of coeval sulfates. This isotopic signal is relatively clearly expressed in rocks as old as 2.3 billion years. The interpretation of the older rock record is controversial, and might indicate that sulfate reducing bacteria arose between 2 and 3 billion years ago.

The biological isotopic signatures for carbon and sulfur differ in that the carbon signal is typified by a clearly biomodal isotopic contrast between the carbonates and organics, whereas the sulfur "biogenic" signal is the wide range of $^{34}S/^{32}S$ values formed at low temperature, most frequently

in sediments. These patterns reflect the properties of the specific environments and organisms that formed them, and, as such, allow us to interpret them as biological signatures.

Because the stable isotopes of carbon and sulfur can reflect their chemistry, they will be useful probes of the Martian surface. Will they show that carbon and sulfur have been reacting at ambient temperatures due to some consistent, life-like process? Perhaps we will be able to measure the average isotopic compositions of carbon and sulfur in the Martian crust (e.g., measured perhaps by analyses of igneous rocks). Can an isotopic contrast between these average values and composite values for carbonates or sulfates be used to infer the presence of other more reduced, carbon and sulfur reservoirs, even at sites where no reduced species are found?

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METHODS AND DECISION MAKING ON A MARS ROVER FOR IDENTIFICATION OF FOSSILS

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We are developing a system for automated fusion and interpretation of image data from multiple sensors, including multispectral data from an imaging spectrometer. Classical artificial intelligence techniques and artificial neural networks are employed to make real time decisions based on current inputs and known scientific goals. Emphasis is placed on identifying minerals which could indicate past life activity or an environment supportive of life.

Multispectral data can be used for geological analysis because different minerals have characteristic spectral reflectance in the visible and near infrared range. Absolute identification of minerals is probably not possible using only multispectral data; mixtures of minerals in one rock, albedo levels, and mineral crystal size alter real spectra from the laboratory results. However, classification of each spectrum into a broad class, based on overall spectral shape and locations of absorption bands is possible in real time using artificial neural networks.

The goal of this system is twofold:

First, multisensor and mutispectral data must be interpreted in real time so that potentially interesting sites can be flagged and investigated in more detail while the rover is near those sites. In particular, we are trying to identify the spectral features that will identify potential life indicators: Carbonates often derive from fossilization of carbon based life forms. Clays may derive from past water activity, thus indicating a good starting point when looking for fossils.

Second, the sensed data must be reduced to the most compact form possible without loss of crucial information. Multispectral data can be very large 200 or more bytes for a single image pixel. Complete data sets are too large to be transmitted to Earth. We are developing methods for real time clustering of spectrally similar pixels using hierarchical neural networks for edge-finding and classification. The multispectral information in an image can represented as a set of compact region descriptors and representative spectra for each region. This description may then be transmitted to Earth for further interpretation by human operators. Currently we expect to realize a 500 fold data reduction.

Autonomous decision making will allow a rover to achieve maximum scientific benefit from a mission. We are considering both a classical rule based approach and a decision neural network for making real time choices. For example, if a potentially interesting region is discovered while the rover is taking a panoramic survey of its surroundings, higher resolution images should be taken and transmitted automatically without awaiting instructions from human analysts.

Decision making is based on incoming data and current scientific goal. Any system must be flexible enough to allow for dynamically changing goals. If the current goal changes from "search for carbonates" to "differentiating between classes of silicates," this will change the wavelengths to be examined most closely and the spectral features considered important.

Neural nets may work well for such adaptive decision making. A rule based decision system might require extensive search through numerous rules covering all possible input states. A neural net can be trained to work in two steps. First, the actual input state (consisting of sensor inputs, outputs of classification and feature detector nets, resolution, distance and scientific goal information) is mapped to the closest of a number of memorised states. The mapping algorithm takes into account that some input parameters (e.g., scientific goal) are more important than others (e.g., a single spectral feature). Then the net produces an output decision based on the matched memory state.

A decision network will allow decision making in real time for any type of situation that can be anticipated in advanced. Rule based methods may be added for handling exceptions, with a facility for requesting guidance from Earth for unusual situations.

Real time, autonomous image data analysis and decision making capabilities are required for achieving maximum scientific benefit from a rover mission. The system under development will enhance the chances of identifying fossils or environments capable of supporting life on Mars.

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DISTRIBUTION OF DESERT VARNISH IN ARIZONA

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Desert varnish is the dark coat of clay and ferromanganese oxides developed on exposed rock surfaces in arid regions. It forms from the accretion of material from windblown dust. The distribution of desert varnish has been mapped in Arizona. It was discovered that desert varnish could be mapped on a regional scale. Well developed desert varnish is common on stable rock surfaces in areas having alkaline soils and less than about 25 centimeters of annual precipitation. Rock surfaces in areas having more than 40 cm of annual precipitation are generally devoid of desert varnish.

An experiment was conducted with varnished desert pavement stones. The stones were broken in half and one half was set on a roof in central Illinois from April until October. Removed from the alkaline desert environment, it only took seven months for the varnish to develop an eroded appearance. This experiment graphically illustrates the dependency of desert varnish on alkalinity. In this context, the zones of eroded desert varnish in Arizona indicate that the area of active desert varnish formation has fluctuated, expanding in drier times and contracting/eroding in wetter times.

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ISOTOPE RATIO MONITORING GAS CHROMATOGRAPHY MASS SPECTROMETRY
(IRM-GCMS)

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On Earth, the ^{13}C content of organic compounds is depleted by roughly 13 to 23 permil from atmospheric carbon dioxide. This difference is largely due to isotope effects associated with the fixation of inorganic carbon by photosynthetic organisms. If life once existed on Mars (and it utilized similar enzymatic and metabolic processes as does life on Earth), then it is reasonable to expect to observe a similar fractionation. Although the strongly oxidising conditions on the surface of Mars make preservation of ancient organic material unlikely, carbon-isotope evidence for the existence of life on Mars may still be preserved. Carbon depleted in ^{13}C could be preserved either in organic compounds within buried sediments, or in carbonate minerals produced by the oxidation of organic material.

A technique is introduced for rapid and precise measurement of the ^{13}C contents (at natural abundances) of individual organic compounds. A gas chromatograph is coupled to an isotope-ratio mass spectrometer through a combustion interface, enabling "on-line" isotopic analysis of isolated compounds. The effluent of an open tube capillary column is split between a flame ionization detector (in order to yield a conventional chromatogram) and a micro-volume combustion system. Hydrogen is used as the carrier gas, and, downstream from the splitter, is removed completed by use of a palladium-tube separator and is replaced by a small flow of helium (0.25 mL/min). Removal of carrier gas allows use of columns with flow rates as high as 20 mL/min. If the bore of the chromatographic column is small enough that the optimum carrier-gas flow-rate is 1 mL/min or less, then the palladium-tube separator can be bypassed with the following modifications: an inert gas (e.g., helium) replaces the carrier gas in the chromatographic column and the split ratio between the combustion interface and the FID must be decreased so that 0–0.5 mL/min (or less) of carrier is delivered to the combustion system. Water produced by combustion is removed by diffusion through a permeable membrane, and peaks of CO_2 are carried directly into the ion-source of the mass spectrometer. Manipulation of small pressure gradients allows systematic control of gas flow, enabling solvent diversion, admission of standards and maintenance of O_2 in the combustion reactor without disturbance of flow to the mass spectrometer. Ion-source characteristics are adjusted to yield maximum sensitivity and linearity; masses 44, 45, and 46 are continuously collected by triple Faraday cups connected to high-speed amplifiers. The isotope rations are determined by integration of ion currents over the course of each chromatographic peak. Software incorporates automatic peak determination, corrections for background, and deconvolution of overlapped peaks. Because input of chromatographic effluent to the combustion system is continuous, all peaks in a chromatogram can be isotopically analyzed from a single injection.

Isotopic ratios of 0.67 nanomole samples of CO_2 introduced upstream from the palladium separator are measured with a standard deviation of better than 0.2 permil. The standard deviation of ratio measurement for samples between 5 and 30 nanomoles is 0.06 permil. In the absence of any correction, observed isotopic composition of the CO_2 peaks decreased by 0.05 permil per nanomole due to ion-source non-linearities. Overall performance of the instrument was evaluated by the

analysis of a mixture of high purity n-alkanes (19-25) of known isotopic composition. Isotopic values measured via IRM-GCMS averaged within 0.55 permil of their conventionally measured values. Repeated measurements (n=5, average sample size = 12 nmol C delivered to ion source) yield an average standard deviation of 0.65 permil for each compound. At present, performance is limited by the accuracy of background corrections and by the method of standardisation. Two-nanomole aliquots of standard CO_2 are introduced between sample peaks. Background corrections are determined for each peak.

There are several possible roles IRM-GCMS might play in the Mars missions, most notable is post-flight analysis of organic samples. In addition, the technology of on-line isotopic determination can be adapted for the analysis of inorganic carbon, for example, in carbonate minerals. Instrumentation for either application could be included for on-board experiments.

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MICROBIAL TRACE FOSSILS IN ANTARCTICA AND THE SEARCH FOR EVIDENCE OF EARLY LIFE ON MARS

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It is possible to hypothesize that, if microbial life evolved on early Mars, fossil remnants of these organisms may be preserved on the surface. However, the cooling and drying Mars probably resembled a cold desert and such an environment is not suitable for the process of fossilization.

The frigid Ross Desert of Antarctica is probably the closest terrestrial analog to conditions that may have prevailed on the surface of the cooling and drying Mars. In this desert, cryptoendolithic microbial communities live in the airspaces of porous rocks, the last habitable niche in a hostile outside environment. The organisms produce characteristic chemical and physical changes in the rock substrate. Environmental changes (deterioration of conditions) may result in death of the community. Although no cellular structures are fossilized, the conspicuous changes in the rock substrate are preserved as trace fossils. Likewise, microbial trace fossils (without cellular structures) may also have been preserved on Mars: Discontinuities in structure or chemistry of the rock that are independent of physical or chemical gradients may be of biological origin. Ross Desert trace fossils can be used as a model for planning search strategies and for instrument design to find evidence of past Martian life.

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THE SEARCH FOR AND IDENTIFICATION OF AMINO ACIDS, NUCLEOBASES AND NUCLEOSIDES IN SAMPLES RETURNED FROM MARS

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INTRODUCTION:

The Mars Sample Return mission will provide us with a unique source of material from our solar system; material which could advance our knowledge of the processes of chemical evolution.

Investigations based on the Viking datasets have shown that geologically primordial Mars was in many biologically important ways similar to primordial Earth: the presence of surface liquid water, moderate surface temperatures, an atmosphere of carbon dioxide and nitrogen, and high geothermal heat flow.[1] Indeed, it would seem that conditions on Earth and Mars were fundamentally similar during the first one billion years or so. As has been pointed out by McKay[1] and others, Mars may well contain the best preserved record of the events that transpired on the early planets. Examination of the early record will involve an extensive search, ranging from microfossils to isotopic abundance data.

We propose an investigation of the returned Mars samples for biologically important organic compounds, with emphases on amino acids, the purine and pyrimidine bases, and nucleosides. These studies would be conducted on subsurface samples obtained by drilling past the surface oxidizing layer with emphasis on samples containing the largest quantities of organic carbon as determined by the rover GCMS.

A. Sample Extraction

Extraction of these molecules from the returned samples will first be performed using the hydrothermal extraction technique described by Cheng and Ponnamperuma[2]. More rigorous extraction methods will be developed and evaluated, as Hayatsu, *et al.*[3] reported improved yields of purines from meteorites with the use of hydrochloric acid. The extract will be analyzed for amino acids, nucleobases and nucleosides.

B. Analysis of Sample Extracts

For analysis of the extract for free amino acids or amino acids present in a bound or peptidic form, aliquots will be analyzed by capillary GCMS both before and after hydrolysis with 6N hydrochloric acid. Establishment of the presence of amino acids would then lead to the next logical step which would be the use of chiral stationary GC phases to determine the enantiomeric composition of the amino acids present, and thus potentially establish their biotic or abiotic origin.

Successful examination of the returned Mars samples for the presence of indigenous amino acids and the determination of their enantiomeric composition will obviously require rigorous exclusion of terrestrial contamination, and our study of the returned lunar samples provides considerable background on matters ranging from sample acquisition, processing and handling to evaluation of the purity of reagents and glassware used.

Confirmational analyses for amino acids would include ion-exchange and reversed-phase liquid chromatographic analyses. For analyses of the returned Mars samples for nucleobases and nucleosides, affinity and reversed-phase liquid chromatography would be utilized. This technology coupled with scanning UV detection for identification, presents a powerful tool for nucleobase and nucleoside analysis. Mass spectrometric analysis of these compounds would confirm their presence in samples returned from Mars.

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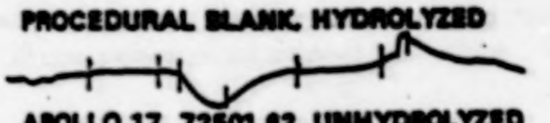

Figure 1. CIE analysis of Apollo 17 lunar fines.

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SOIL DEVELOPMENT IN POLAR DESERTS: IMPLICATIONS FOR EXOBIOLOGY AND FUTURE MARS MISSIONS

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Chemical alterations, weathering and diagenesis of soil profiles from the Dry Valleys of Antarctica have been studied as analogs of regolith development for the Martian regolith. Chemical weathering processes play an important role in soil development within the Dry Valleys of Antarctica[1]. The present study has focused on a suite of core samples taken within the valley floors in addition to samples taken in the vicinity of evaporite and brine ponds. Analyses of water-soluble cations and anions from core samples have been carried out along with petrographic analysis of selected samples. Study of the water-soluble ions has shown that ionic transport processes operate primarily above the permanently frozen zone. Abundances of the water-soluble ions reflect the nature of secondary minerals produced by evaporation and weathering processes.

Chloride, calcium and sodium abundances for soils from the cores within the North and South Forks of Wright Valley, reflect the secondary mineralogy within the soil columns. Sodium occurs primarily as halite ($NaCl$) while chloride ions are associated with both halite and antarcticite ($CaCl_2 \cdot 66H_2O$). Calcium ion abundances reflect phases such as gypsum, calcite and antarcticite. Chloride abundances ranged from as low as 10 micromoles for soils from South Fork evaporite ponds to values as great as 4,000 micromoles in salt-rich evaporite layers from the edge of Don Juan Pond. In common with soils from other arid parts of the world, high soluble salt concentrations are characteristic of Dry Valley soils and core samples. The water-soluble ions from the soils can assist in the determination of the nature of the secondary mineralogy. Mass balance calculations for Na, Ca and Cl abundances in soils reflect the appearance of halite and antarcticite. In areas where excess Ca is present, XRD studies show the presence of gypsum.

It is well known that the Martian surface conditions may be favorable for chemical weathering.[2] Primary silicates would be expected to be reactive with any ground water. Because of the possible existence of an extensive subsurface system of water-ice and maybe even liquid water just below the Martian surface,[3] it seems likely that water is available to assist in the weathering of the primary minerals. Such weathering could result in the formation of clays, sulfates, carbonates, hydrates, halides, and zeolites. The study of the Dry Valley cores has shown that they may be excellent analogs to weathering processes operating on the near-surface of Mars. Since movement of water within the near-surface region clearly results in chemical weathering, leaching and salt formation in the Dry Valleys, similar processes are probably operating within the Martian regolith. Any experiment performed *in situ* on the Martian surface must take into account the presence of salts.

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ANCIENT LAKES ON MARS?

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The valley systems in Mars' ancient cratered terrain provide strong evidence for a warmer and wetter climate very early in the planet's history. Examination of Viking orbiter images shows that the valley systems in some instances debouch into closed depressions that could have acted as local ponding basins for flow. Ancient craters, for example, can provide such a setting. A survey of Mars' equatorial region using USGS 1:2,000,000 photomosaics shows that numerous local depressions at the confluence of valley systems exist. These depressions are typically of the order of ~ 100 km in size, and are characterized by many valleys flowing into them and few or none flowing out. If flow ponding did take place, these basins would have contained lakes for some (perhaps brief) period during Mars' early warmer epoch.

Although the collection basins are numerous, location of ones that have not suffered significant subsequent geologic modification is difficult. When examined in detail, many basins are found to exhibit morphologic features (such as "wrinkle ridges") which suggest that volcanic lavas may have filled them subsequent to any early fluvial activity. Many Martian lavas had very low viscosities, and the lava, like the water, tended to flow into locally depressed regions. For this reason, the basins that exhibit possible volcanic features have not been given high priority in our mapping efforts. It is worth nothing, however, that subsequent cratering would be very effective at punching through lava units and excavating whatever lies underneath.

Two detailed maps of valley systems and local ponding basins in USGS 1:2,000,000 sub-quadrangles have been completed and a third is in progress. The completed regions are in Mare Tyrrhenum (MC-22 SW) and Margarifter Sinus (MC-19 SE), and the region in progress is in Iapygia (MC-21 NW). On these maps (to be exhibited at the meeting), the valley systems and interpreted margins of ponding basins have been indicated. Some of these margins are the rims of ancient craters, while others have irregular shape and are simply local closed depressions among surrounding craters. The floors of the basins are generally very smooth as seen in Viking orbiter medium resolution images. In one case, however, the floor deposits have a complex hummocky topography which resembles features (such as patterned ground and pingoes) formed on Earth from multiple freeze-thaw of water-saturated sediments.

From a geological perspective, these depressions are of interest for two reasons. First, regardless of whether the water that drained into them formed long-lived lakes or ephemeral playas, the depressions were surely the sites in which the materials eroded from the valleys were deposited. The physical nature of such sediments could preserve important information about the physical conditions that existed in the basins at the time of deposition. Second, the chemical composition of the sediments could preserve evidence of water-atmosphere interactions during the early period of Martian climate. Atmospheric carbon dioxide would dissolve in the water, and would react with cations brought in by the inflowing streams. The cations would react with the hydrated carbon dioxide to form solid carbonate minerals (e.g., $CaCO_3$) that would tend to precipitate out of solution to form carbonate sedimentary deposits. Formation of carbonates in this manner might account for some of the CO_2 lost from the early more dense atmosphere.

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MINERALOGICAL SINKS FOR BIOGENIC ELEMENTS ON MARS

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INTRODUCTION. The efficacy of biochemical reactions on Mars should depend not only on concentrations of the biogenic elements[1] H, C, N, O, and S but also on the forms (compounds and water-soluble ions) that are available to those elements. It is possible that mineralogical reactions could act to lock biogenic elements into relatively inaccessible inorganic forms or, alternatively, to shelter sensitive organic compounds from chemically hostile environments. Recognition of these competing pathways is essential in planning sampling missions and *in situ* experiments directed toward assessing the biological potential of Mars.

SINKS THROUGH CHEMICAL WEATHERING. The four principal types of chemical weathering expected for Mars are oxidation, hydration, carbonation, and solution.[2] *Oxidation* irreversibly (with respect to reverse reactions under most natural conditions) locks oxygen into oxides and silicates. In general, the oxidant can be molecular oxygen, water, or carbon dioxide. *Hydration* refers strictly to reaction of molecular water to form hydrated oxides, silicates or salts whereas *carbonation* consists of reactions involving carbon dioxide to form carbonate minerals. Given suitable volumes of liquid water, *solution* proceeds by either congruently or incongruently dissolving pre-existing minerals to form various simple or complex ions, ultimately with precipitation of new phases.

SINKS THROUGH SORPTION AND ION EXCHANGE. In contrast with chemical weathering reactions, sorption and ion-exchange reactions can sequester biogenic chemical species (and their antagonists) through contact with independently formed mineral substrates. The major available sorbents are Fe-, Mn-, or Al-oxyhydroxide minerals (and their gel equivalents), layer-structured silicates ("clay" minerals), and zeolites. Ferric oxyhydroxides sorb Mn and foster disproportionation reactions among Mn-oxides, producing strongly oxidizing surface layers[3] that might be destructive to organic compounds. Phyllosilicates and zeolites exhibit preferential sorption of organic compounds that can include either preservation or catalyzed decomposition of those compounds.[4] Sorbed species can be released by later exchange reactions.

EXAMPLES OF MECHANISMS AND RATES: ANTARCTIC METEORITE WEATHERING. Many of the sinks that are likely to be available on Mars currently operate in analogous fashion in the glacial and periglacial weathering environments that affect meteorites found in Antarctica. Under mostly sub-freezing conditions and in the presence of only limited quantities of liquid water, mafic-igneous meteorites have experienced incipient clay-mineral formation in less than 1 million years.[5] Both the clay mineraloids and associated ferric rust have sorbed large concentrations of carbon, sulfur, potassium, boron, and water through essentially nonbiological processes. Weathered basaltic achondrites, in particular, might be useful test cases for assessing the behavior of biogenic elements under ostensibly abiotic, Mars-analogous conditions.

SEARCH STRATEGY FOR MARS. Sampling and analysis of Martian materials for exobiology should not be restricted to carbonate-bearing units but should extend to units that contain clay mineral(oid)s and zeolites—if such materials exist on Mars. Aluminosilicate sorbents are the most likely phases to have trapped and preserved organic compounds. If ferric-oxide-related oxidants are important on Mars, then units containing the least rust might be relatively more hospitable to organic compounds than would common, rusty surface fines.

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A CORE HANDLING DEVICE FOR THE MARS SAMPLE RETURN MISSION

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This paper reports on an ongoing project at the University of Wisconsin, Madison, to design a core handling device for use on Mars. In addition to this design effort, there is currently activity at UW on several other aspects of the Mars rover including the sample return canister, the core drill and the mobility systems.

To provide a context for our design study we have assumed that a Mars Rover/Sample Return Mission would have the following characteristics:

- A year or more in length.
- Visits by the rover to many sites, on the order of 50 or more.
- Many cores being drilled by the rover, on the order of 100 or more. Each core being about a meter long.
- The capability of returning about 5 kg of Mars regolith to Earth.

These characteristics lead us to believe that in order to bring back a variegated set of samples that can address the range of scientific objectives for a MRSR mission to Mars there needs to be considerable analysis done on board the rover. Furthermore, the discrepancy between the amount of sample gathered and the amount to be returned suggests that there needs to be some method of choosing the optimal set of samples.

This type of analysis will require pristine material—unaltered by the drilling process. Since the core drill thermally and mechanically alters the outer diameter (about 10%) of the core sample, this outer area can not be used. The primary function of the core handling device is to extract subsamples from the core and to position these subsamples, and the core itself if needed, with respect to the various analytical instruments that can be used to perform these analyses.

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MARS, CLAYS, AND THE ORIGINS OF LIFE

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An outstanding problem that the scientists and engineers of NASA faced in designing the Viking mission to Mars could be phrased in simple terms: How to detect life in the Martian soil? The tests that were decided upon were designed to look for respiration and photosynthesis. The respiration experiment or labeled release (LR) experiment consisted of adding organic molecules such as formate to the Martian soil and measuring the carbon dioxide released. The results of this experiment were positive. The photosynthetic experiment or pyrolytic release (PR) experiment consisted of adding water to a Martian soil sample in a Martian atmosphere of carbon dioxide and nitrogen and shining light on it and measuring the organic molecules formed. The results of this experiment were also positive. Thus both tests for life in the Martian soils were positive. However, when the measurement for organic molecules in the soil of Mars was made, none were found. The interpretation given is that the inorganic constituents of the soil of Mars were responsible for these observations. The inorganic analysis of the soil was best fitted by a mixture of minerals: 60-80% clay minerals, iron oxide, quartz and soluble salts such as halite (NaCl). The minerals most successful here on Earth in simulating the PR and LR experiments are iron-rich clays (Banin and Margulies, 1983).

There is a theory that considers clays as the first organisms capable of replication, mutation and catalysis and hence of evolving (Cairns-Smith and Hartman, 1986). Clays are made up of various ions imbedded in a "two-dimensional" silicate lattice. The ions are mainly silicon, aluminum, iron and magnesium. Clays are formed when liquid water causes the chemical weathering of rocks. The concentration of ions in clays, e.g., iron and magnesium, is extremely variable and thus clays can be considered solid solutions. In 1966, Cairns-Smith (1966) proposed that the original genes were clays. The distribution of ions such as aluminum, magnesium and iron played the role of bases in the DNA. The information was stored in the distribution of ions in the octahedral and tetrahedral layers. The major idea is that clays could not only adsorb and catalyze reactions between organic molecules, but that they could, like DNA or RNA, replicate. Thus, two sheets of clay would be like the two complementary strands of a DNA molecule. When they replicated, each sheet of clay would be a template for a new sheet. The ion substitutions in one clay sheet would give rise to a complementary or similar pattern on the clay synthesized on its surface. If we now suppose that, as with DNA, an error of replication or mutation is possible, then replicating clays could evolve. It has been theorized that it was on the surface of replicating iron-rich clays that carbon dioxide would be fixed in the light into organic acids such as formic or oxalic acid (Hartman 1975).

If Mars had liquid water during a warm period in its past, clay formation would have been abundant. These clays would have replicated and evolved until the liquid water was removed due to the cooling of Mars. It is entirely possible that the Viking mission detected life on Mars, but it was clay life that awaits the return of water to continue its evolution into life based on organic molecules.

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SNOW AS A HABITAT FOR MICROORGANISMS

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There are three major habitats involving ice and snow, and the microorganisms studied from these habitats are mostly eukaryotic. Sea ice is inhabited by algae called diatoms, glacial ice has sparse populations of green algae called desmids, and the temporary and permanent snows in mountainous regions and high latitudes are inhabited mostly by green algal flagellates. It is the latter habitat that I study and that will be emphasized here. Light is an important factor regulating the distribution of individual species of these algal flagellates. In response to high-intensity light, snow algal cells may produce yellow, orange or red secondary carotenoid pigments. Thus snowfields containing these algae may appear red (open exposure), yellow to orange (partial shading) or green (maximum shading). Red snow is easily seen, but orange and green snows are more difficult to locate and can be easily overlooked in the snowpacks. Several groups of algae are known from snow, and dominant species worldwide belong to the photosynthetic green algal flagellates, *Chloromonas* and *Chlamydomonas*. Other groups include heterotrophic euglenoids and photoauxotrophic golden algae. Life cycles of green algal flagellates are short-lived, complex and governed by light, nutrients, temperature and snowmelt. Active growth occurs during spring and summer when air temperatures remain above freezing for several days. This allows for liquid water to surround snow crystals in snowbanks that are 0.2–1.5 m deep. Light penetrates through the snow germinating the resting spores that lie beneath the snowpack. Resting spores release flagellate cells that swim in the liquid water surrounding the snow crystals at 0° C and migrate towards the surface of the snowpack. Nutrition for growth is derived from the soil at the time of germination, from nutrients released from snow crystals, and from debris and dust from the snowbank surface. The rapid utilization of nutrients by the algae forces the life cycle into the production of resistant resting spores before the snow melts. Resting spores formed through sexual and asexual processes may withstand long periods of desiccation, varying air temperature, and (or) high levels of irradiation after the snow is gone.

A culture collection of approximately 100 strains of snow algae is being maintained at Colgate University. Using defined media, species of snow algae studied axenically in the laboratory indicate that nutrients, pH, temperature, light and alleopathic effects from conifers may influence their growth. Species of snow algae studied in the laboratory may utilize either inorganic or organic sources of phosphorus and nitrogen for their growth. In the Adirondack Mountains, New York, green snow was not discovered until the early 1970's. This is interesting because these algae have been overlooked for two centuries in a region where there are many naturalists and amateur botanists. We are still discovering microorganisms on this planet, and only recently we have found two new groups of prokaryotes, the chloroxybacteria or prochlorons (1975) and *Heliobacterium* (1983). Thus we should not give up our inquiries into the possibility of life on the planet Mars when we have evidence of still finding new organisms on planet Earth. In the Adirondacks, acid precipitation may be selecting for acidic strains of snow algae that grow optimally at pH 4.0–5.0. Populations of *Chloromonas* affect snow chemistry through metabolic processes, and red snow caused by the green alga, *Chlamydomonas nivalis*, accumulates trace metals many hundreds to thousands of times greater in the cells than what is found in the surrounding snow. The snow alga, *Chloromonas* *pichinchae*, grows optimally at temperatures near freezing (1–4° C), and warmer temperatures (10° C) may alter cell morphology and cause death of these cells. One species of *Chlainomonas* loses its flagella at temperatures above 4° C as observed using a cooling stage. Most species of snow algae develop lipids in response to subfreezing temperatures, but some lyse under these conditions. The snow alga, *Raphidonema nivale*, grows optimally over a wide temperature range from 1–15° C, and other species of snow algae grow best at more mesophilic temperatures. Higher light intensity causes cells of *Raphidonema nivale* to divide more frequently, and cells are more elongate when grown under low light intensity. One species of the flagellate, *Chloromonas*, grows optimally in shaded snowbanks several centimeters beneath the surface receiving about one-thirtieth incident irradiation. Conifers, which grow near snowbanks containing algae, may stimulate or inhibit algal growth through the release of chemical compounds into the snow. In summary, some species of snow algae grow under extreme conditions of cold temperature (0° C), high levels of irradiation, and/or high acidity (optimizing at pH levels near 4.0).

In the snow ecosystem, other organisms encountered besides algae include bacteria, fungi, lichen pieces, protozoa, rotifers and other invertebrates. Most of these organisms have not been studied critically, and little is understood concerning the interactions between them or with the algae. Encapsulated bacteria have been found in the cell walls of the snow alga, *Chlamydomonas nivalis*. It is not known how these bacteria grow in snow or whether there is a symbiosis between the two organisms. Hyphomycetous fungi are known from surface snow; their development is not clear, and it is not known if they exchange nutrients passively with snow algae. The snow fungus, *Phacidium*, grows through melting snow, and algae may passively adhere to the surfaces of the fungal hyphae. When the snow melts, strands of dried fungi with adhering algae may be distributed elsewhere by wind. Several species of protozoa and rotifers are primary consumers of snow algae selecting green colored cells over those that are orange or red. In melting snowbanks, these consumers are usually found in small surface depressions saturated with liquid water. Detritus eaters in the snow include species of waterbears and nematodes. Other species of animals may include snowworms, insects and arachnids.

In conclusion, it is not likely that the eukaryotic snow algae presented here are candidates for life on the planet Mars. Evolutionarily, eukaryotic cells as we know them on Earth may not have had the opportunity to evolve on Mars (if life evolved at all on Mars) since eukaryotes did not appear on Earth until almost two billion years after the first prokaryotic organisms. However, the snow/ice ecosystems on Earth present themselves as extreme habitats where there is evidence of prokaryotic life (eubacteria and cyanobacteria) of which we know literally nothing. Is it possible that some form of life may be locked up in the ice water near the polar regions of Mars? Any future surveillances of extant and/or extinct life on Mars should include probes (if not landing sites) to investigate sites of concentrations of ice water (?liquid water). The possibility of signs of life in Martian polar regions should not be overlooked.

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CHEMICAL EVOLUTION AND THE PRESERVATION OF ORGANIC COMPOUNDS ON MARS

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Several lines of evidence suggest that the environment on early Mars and early Earth were very similar.[1] Since life is abundant on Earth, it seems likely that conditions on early Earth were conducive to chemical evolution and the origin of life. The similarity between early Mars and early Earth encourages the hypothesis that chemical evolution might have also occurred on Mars, but that decreasing temperatures and the loss of its atmosphere brought evolution to a halt. The question then arises: Can one expect to find on Mars remnants of organic material dating back to this early clement period?

In an attempt to answer this question a literature search was undertaken ranging from organic geochemical studies to stability measurements of selected organic compounds. In many instances analysis of the organic content of ancient sediments has revealed the presence of minute amounts of hydrocarbons, fatty acids, amino acids and other organic material, most likely of biological origin. Even though it cannot be unequivocally demonstrated that the organic material found within the one billion years old cherts is syngenetic with the associated sediments,[2] stability studies in the laboratory indicate that amino acids and other biochemically interesting compounds could be quite stable over geological time scales.[3] Because of Mars' geological stability, the now prevailing lower temperatures and absence of liquid water, organic matter could have been preserved on Mars better than on Earth. Nevertheless, no organic material has been found on Mars to date.[4] The absence of organics on the Martian surface may well be accounted for by its extensive irradiation by UV, which would certainly destruct any organic compound present. Therefore, the absence of organic material on the surface does not preclude the presence of it deep inside the planet where UV radiation cannot reach. Accordingly, if chemical evolution leading to the formation of organic material had occurred on early Mars, traces of it should be found beneath the Martian surface.

Hence the discovery of any organic compound on Mars would be highly significant for Exobiology. On the other hand, if no organic compounds are found on Mars, this may force a reassessment of the theories of chemical evolution and the origin of life on Earth.[5] Therefore, extensive sampling and detailed analysis for organic constituents in selected sites beneath the Martian surface is a primary objective for Exobiology.

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THE VIKING BIOLOGY RESULTS

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A brief review of the purposes and the results from the Viking Biology experiments is presented here, in the expectation that the lessons learned from this mission will be useful in planning future approaches to the biological exploration of Mars. Considerable discussion, prior to the selection of the final Biology instrument payload, resulted in the conclusion that a single concept would not be adequate, since so little was then known about potential micro-environments on Mars. This resulted in the final inclusion of three different experiments in the Viking mission, each one based on different assumptions about what Martian organisms might be like. In addition to the Viking Biology Instrument (VBI), important corollary information was to be obtained from the Viking lander imaging system and from the molecular analysis experiments that were to be conducted using the Gas Chromatograph-Mass Spectrometer (GCMS) instrument.

After examining upwards of 5,000 pictures from the lander imaging instrument, no "biological" objects were noted at the lander sites; no color changes, attributable to living organisms were seen; and—aside from nominal changes due to weather—no objects where observed to move into or out of the fields of view.

The GCMS instrument, which could detect organic compounds down to a few parts per billion (for molecules containing three or more carbon atoms) and down to a few parts per million (for smaller organics), did not detect any organic compounds.

The VBI consisted of three basic experimental components packaged into a volume of about 1 ft^3 and weighing about 35 lbs. Using all of the experimental modes, it was possible to incubate Martian "soil" (in the dark or with simulated solar illumination; wet, dry, or with a humid atmosphere; and with and without added nutrients) for periods of up to several months. In all, 26 experiments were conducted at the two sites. To distinguish between biological and non-biological signals, in some experiments, the "soil" was heated to "sterilizing" temperatures (145° to 175° C) prior to incubation.

The Gas Exchange experiment (GEX) measured gas changes in the headspace of the incubation chamber containing Martian "soil" samples. In the "humid" mode of the GEX, the samples were incubated without nutrients, but in a humid atmosphere. This experiment, which was performed three times, showed a very rapid evolution of oxygen upon humidification. Furthermore, the reaction, which was stable to sterilization and to storage at spacecraft temperatures for about 5 months, appears to be inversely correlated with the water content of the samples. Subsequent contact of the samples with a nutrient solution consisting of a rich aqueous mixture of organic compounds did not result in the evolution or uptake of gases beyond that expected from non-biological processes (desorption of atmospheric gases; reactions between the atmospheric components and nutrients).

In the Labeled Release (LR) experiment, soil samples were incubated with a dilute aqueous solution of simple organic compounds labeled with ^{14}C and the headspace monitored for the release of labeled

CO_2. Each time samples were tested by the LR experiment, there was a rapid release of labeled gas (amounting to about 10–15% of the added counts), followed by a period during which labeled gas continued to be released at a very slow rate for up to several weeks. The rapid evolution of label was abolished by sterilization temperatures; was reduced by about 70% by prior heating at about 45° C; and was lost upon storage at spacecraft temperatures.

The Pyrolytic Release (PR) experiment tested for the uptake of either ^{14}CO or $^{14}CO_2$ into organic matter during incubation of samples in the light or in the dark. Weak "positives" were obtained—indicative of the fixation into organics of one or the other of the added compounds—seven of the nine times that the PR was performed, including one in which the sample had first been heated to 90° C. Taken as a whole, the Viking data yielded no unequivocal evidence for a Martian biota at either landing site. The results also revealed the presence of one or more reactive oxidants in the surface material and these need to be further characterized, as does the range of micro-environments, before embarking upon future searches for extant life on Mars.

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ECOLOGICAL CONSIDERATIONS FOR POSSIBLE MARTIAN BIOTA

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Current climatic and geological evidence suggests that, like early Earth, conditions on ancient Mars may also have been favorable for the origin and evolution of life. The primordial atmospheres of the two planets were quite similar, composed primarily of CO_2, N_2, and water vapor at a total atmospheric pressure of ~ 1 bar. Each of these gases are important for the evolution of biological systems as we know them. Although there was no absolute confirmation of organic carbon in the regolith, the influx of organic material from meteorites and the production of organic material under weakly reducing atmosphere (albeit difficult), similar to early Earth, should have allowed some prebiotic synthesis. Organics should not have been in shorter supply on Mars than on Earth. One of the most crucial environmental factors limiting life and biochemical processes is the presence of adequate supplies of liquid water. Out flow channels and valley networks, suggest that abundant liquid water once flowed across the surface of Mars. The abundance of liquid water implies that the temperature of the Martian surface must have been > 0° C and therefore conducive for rapid prebiotic chemical reactions which may have led to the evolution of a living system. The higher temperature could have been maintained by geochemical cycling of CO_2 in and out of the atmosphere via the regolith (as carbonates) long enough for life to have developed on Mars. It is feasible that a thin layer of SO_2 soil from volcanic activity could have somewhat shielded a possible early Martian biota from ultraviolet radiation.

With the exception of nitrogen, there seems to have been a sufficient supply of the biogenic elements (CHOPS) on early Mars for life to have evolved. It has been postulated that primordial Mars contained only 18 mb of nitrogen in the form of N_2 given that only fixed nitrogen is utilized by living systems. Would this have been a sufficient deterrent to cease the evolution of a biological entity? Data we have gathered in the laboratory indicates that there was sufficient nitrogen in the atmosphere to allow biological fixation to occur. Under a total pressure of 1 bar, nitrogen fixing organisms were grown in nitrogen free medium under various partial pressures of dinitrogen (pN_2 1–780 mb). The data suggest that organisms grow at pN_2's of 18 mb or less, although the biomass and growth rates are decreased. The calculated *in vivo* Km's ranged from 46 mb to 130 mb. If organisms adapted on Earth to a pN_2 of 780 mb are capable of growing at these low partial pressures, it is conceivable that nitrogen was not the limiting factor in the evolution of life on early Mars.

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A SEARCH FOR BIOGENIC TRACE GASES IN THE ATMOSPHERE OF MARS

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The detection of certain trace gases in the atmosphere of Mars may serve as a possible indicator of microbial life on the surface of Mars. Candidate biogenic gases include methane (CH_4), ammonia (NH_3), nitrous oxide (N_2O), and several reduced sulfur species. Chemical thermodynamic equilibrium and photochemical calculations preclude the presence of these gases in any measurable concentrations in the atmosphere of Mars in the absence of biogenic production. A search for these gases utilizing either high resolution (spectral and spatial) spectroscopy from a Mars orbiter, such as the Observer, and/or *in situ* measurements from a Mars lander or rover, is proposed.

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THE GEOLOGICAL RECORD OF LIFE 3500 Ma AGO: COPING WITH THE RIGORS OF A YOUNG EARTH DURING LATE ACCRETION

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Thin cherty sedimentary layers within the volcanic portions of the 3,500 to 3,300 Ma-old Onverwacht and Fig Tree Groups, Barberton Greenstone belt, South Africa, and Warrawoona Group, eastern Pilbara Block, Western Australia, contain an abundant record of early Archean life. Five principal types of organic and probably biogenic remains and/or structures can be identified (Lowe, 1986): (1) stromatolites, (2) stromatolite detritus, (3) carbonaceous laminite or flat stromatolite, (4) carbonaceous detrital particles, and (5) microfossils.

Early Archean stromatolites have been reported from both the Barberton and eastern Pilbara greenstone belts. Systematic studies are lacking, but two main morphological types of stromatolites appear to be represented by these occurrences. The Barberton stromatolites (Byerly *et al.*, 1986), which are developed in thin cherty units interbedded with komatiitic lavas in the uppermost part of the Onverwacht Group, and stromatolites in the Towers Formation of the Warrawoona Group (Walter *et al.*, 1980) appear to represent the same type of small, low-relief, unbranched stromatolites. In Barberton, this type of stromatolite appears to have developed preferentially on hard substrates along moderate to low energy rocky coasts. Although some stromatolites occur within units containing replaced evaporites, there is no direct interbedding of evaporites and stromatolites. There is no evidence that evaporitive precipitation exerted a significant control on stromatolite morphology although shoreline splash-type wetting and evaporation may have locally contributed to stromatolite build-up and early lithification.

In both Barberton and Pilbara sequences, these stromatolites are associated with distinctive units of stromatolite-chip breccia. These include beds and lenses from a few mm to over 3 m thick composed of sand- and granule-sized, curved, laminated, sometimes carbonaceous stromatolite plates. Similar detritus is also present within cherty sedimentary units throughout the Onverwacht and Warrawoona Groups that are not otherwise known to contain stromatolites. The ubiquitous association of stromatolites and units of rigid stromatolite debris indicates that the stromatolites were partially lithified during growth but still sufficiently fragile to be easily broken up by wave or current activity. The abundance of stromatolite debris suggests that stromatolites were widely developed along wave-agitated rocky shorelines during accumulation of these early Archean greenstone belt volcanic sequences.

A second stromatolite morphology is developed in the Strelley Pool Chert in the upper part of the Warrawoona Group (Lowe, 1980). Small conical stromatolites are interbedded with silicified evaporites over hundreds of square km and show clear evidence that stromatolite growth was strongly influenced if not controlled by precipitative processes.

Carbonaceous laminite, massive black carbonaceous chert, and banded carbonaceous cherts are common within interflow sedimentary layers in these early Archean greenstone belt volcanic sequences. Most of these layers were deposited under quiet, low-energy, subaqueous conditions. In shallow-water sections, they appear to include both *in situ* silicified bacterial mats and detritus eroded from them. Carbonaceous matter in deeper-water deposits consists exclusively of fine-grained pelagic, hemipelagic, or current-deposited detritus.

Preserved early Archean stromatolites and carbonaceous matter appear to reflect communities of photosynthetic cyanobacteria inhabiting shallow, probably marine environments developed over the surfaces of low-relief, rapidly subsiding, simatic volcanic platforms. The overall environmental and tectonic conditions were those that probably prevailed at Earth's surface since the simatic crust and oceans formed sometime before 3,800 Ma. Recent studies also suggest that these early Archean sequences contain layers of debris formed by large-body impacts on early Earth (Lowe and Byerly, 1986, 1988; Lowe *et al.*, 1988). If so, then these early bacterial communities had developed strategies for coping with the disruptive effects of possibly globe-encircling high-temperature impact vapour clouds, dust blankets, and impact-generated tsunamis. It is probable that these early Archean biogenic materials represent organic communities that evolved long before the beginning of the preserved geologic record and were well adapted to the rigors of life on a young, volcanically active Earth during late bombardment. These conditions may have had parallels on Mars during its early evolution.

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THE NITROGEN CYCLE ON MARS

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Abstract:

Nitrogen is an essential element for the evolution of life as we know it, because it is found in a variety of biologically important molecules. Therefore, N is an important element to study from an exobiological perspective. In particular, "fixed nitrogen" is the biologically useful form of nitrogen. Fixed nitrogen is generally defined as NH_3, NH_4^+, NO_2, or N that is chemically bound to either inorganic or organic molecules, and releasable by hydrolysis to NH_3 or NH_4^+. On Earth, the vast majority of nitrogen exists as N_2 in the atmosphere, and not in the fixed form. On early Mars the same situation probably existed. The partial pressure of N_2 on early Mars is thought to have been 18 mb, significantly less than that of early Earth. Dinitrogen can be fixed abiotically by several mechanisms. These mechanisms include thermal shock from meteoritic infall and lightning, which could produce NO at a rate of $\sim 10^{15}$ molecules J^{-1} in a primitive Martian atmosphere (1 bar total pressure; $CO_2:N_2=33$), as well as the interaction of light and sand containing TiO_2 which produces NH_3 that would be rapidly destroyed by photolysis and reaction with OH radicals. These mechanisms could have been operative on primitive Mars. Any NO produced under these conditions reacts with H forming HNO. In the Martian ocean HNO would react to form $N_2O_2^-$, $N_3O_3^-$, and their conjugate acids. These species would decay leaving a nitrogen pool in the oceans of NO_2^- and NO_3^-. In addition, there may have been organic nitrogen compounds available in the environment. These compounds then would have formed sediment, which may have been buried by volcanic lava to a depth at which gaseous products would be produced and released to the atmosphere. If this burial process did not occur, or was insufficient to produce enough heat and pressure, then any nitrogen that was fixed would remain buried on Mars, and not recycled. Would the relatively low abundance of nitrogen, compared to primitive Earth, have an impact on the origin and early evolution of a living system on Mars? Data gathered in our laboratory suggest that the low abundance of nitrogen alone may not significantly deter the origin and early evolution of a nitrogen utilizing organism. However, the conditions on current Mars with respect to nitrogen are quite different, and organisms may not be able to utilize all of the available nitrogen.

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Mars 94 Mission: Current Plans and Science

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The Soviet Space Program proposes large-scale investigations of Mars as one of the most important trends for the nearest 10–15 years. The objectives include global studies of the surface of Mars, its atmosphere, and the return of soil samples to Earth. The first stage of the program is to be implemented in the mid-90's. It includes measurements from an orbiter and a balloon in the atmosphere and on the surface from a rover as well. This can be done using the high energy upper stage of the launch vehicle. The payload mass could amount to about 1500–1700 kg.

This presentation discusses the capabilities of the Mars Mission in 1994. As presently planned, Mars will be studied concurrently with the following facilities:

- an orbiter with instruments for remote sensing from a polar orbit
- a balloon deployed in the Martian atmosphere (for studies of the atmosphere and surface)
- a rover on the surface (studies of the surface)
- a network of smaller stations on the surface (global meteorological studies)
- a subsatellite (studies of the Mars gravitational field)
- a device to return a container with photo films of the Martian surface taken with a super high resolution (to test a cargo return from the orbit of Mars).

If the exploration of Mars with the MARS-94 spacecraft begins late in 1994 the American Mars-Observer will still be functioning in the near-Mars orbit. Thus, joint operation of the two satellites becomes possible providing coordinated exploration, the creation of joint data banks, joint data interpretation and the development of a joint engineering model of Mars for its further exploration.

There is an opportunity for experiments and instrumentation on the Soviet missions that address questions of interest to exobiology. We invite American scientists to participate in the upcoming Soviet missions, and lend their expertise to the further understanding of the relationship between the physical and chemical evolution of the solar system and the appearance of life.

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ARE THERE CARBONATE DEPOSITS IN THE VALLES MARINERIS, MARS?

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The interiors of the Valles Marineris canyon system contain plateaus of horizontally-layered material where individual layers are laterally continuous over tens of kilometers. Several workers have suggested that they were deposited in lakes that existed in these depressions early in Martian history. Recently, Nedell *et al.* (1987) conducted a detailed study of the Valles Marineris layered deposits using late Viking high-resolution images. These studies show that the deposits form thick sequences of rhythmically layered material whose bases are in the lowest elevations of the canyon floors and whose tops are commonly within a few hundred meters of the surrounding plateaus. Most of the deposits occupy the central canyons, which include Hebes, Ophir, Candor, and Melas chasmata. From stratigraphic relationships, Nedell *et al.* (1987) concluded that the layered deposits formed during roughly the same epoch in which the original tectonic canyons were enlarged by ground ice removal and collapse. Later, the deposits were eroded in some locations, producing their present geometry. This erosional episode may coincide with the formation of the large outflow channels that emanate from the east end of the Valles Marineris. Nedell *et al.* (1987) concluded that deposition in standing water was the only mechanism that could readily explain the distribution, lateral continuity, horizontality, great thickness, and rhythmic nature of the deposits.

If standing bodies of water formed in the Valles Marineris, and an initial thick CO_2 atmosphere had thinned resulting in lower temperatures, these lakes would almost certainly have been ice-covered. We suggest that a considerable fraction of the possible Martian paleolake sediment could be carbonate material that was precipitated in standing water under conditions of high atmospheric pressure of CO_2.

One major problem with carbonate formation on early Mars is maintaining significant bodies of liquid water after the mean temperature fell below 273 K. According to the model of Pollack *et al.* (1987), this would occur when the atmospheric pressure drops below a value of a few bars. Ice-covered lakes fed by transitory surface melting in the Valles Marineris could have provided a stable body of liquid water that would have also had an enhanced level of atmospheric gases. This hypothesis is supported by our observations in the Antarctic dry valleys where perennially frozen lakes contain dissolved atmospheric gases at 200–300% above the equilibrium level and the mean annual temperature is 253 K. By analogy with those lakes, we suggest that lakes in the canyon system could have contained liquid water long after the mean temperatures on the surface were below freezing.

Using data from perennially-frozen Antarctic lakes, McKay *et al.* (1985) developed a general energy balance model for determining the thickness of ice on perennially-frozen lakes. We can apply this model to Martian paleolakes by assuming that ablation, dust loading and optical ice properties are the same as in the nominal Antarctic lake model of McKay *et al.* (1985), and by using the annually averaged sunlight on the equator of Mars, including the lower solar luminosity of the early sun (see e.g., Pollack *et al.*, 1987) and attenuation atmospheric dust arbitrarily chosen to have an optical depth of 0.5. The resulting value of the solar flux is 126 W m^{-2}. This yields an ice thickness of 3.4 m when the mean temperature is 250 K, and 11 m and 19 m for mean temperatures of 240 K and 230 K, respectively. These values for the ice cover compare to the Antarctic lake ice cover thicknesses of 4–6 m. For the same surface temperature, Mars lakes would have thinner ice covers than Antarctic lakes due to the fact that the mean insulation on the primordial equatorial Mars was greater than in Antarctica today (104 W m^{-2}). Ice covers on Mars would have been much thicker if ablation rates in a thinning Martian atmosphere were below the Antarctic values (McKay *et al.*, 1985).

Snowmelt and groundwater flow into the lakes would have carried in CO_2 and cations leached from the adjacent rocks. The ions and gases would have been concentrated in the lake water due to dissolution upon freezing. Thus the process of inflow of water, freezing, followed by ablation at the ice surface would have concentrated gases and ions in the water column and enhanced carbonate precipitation. This mechanism is known to operate in the Antarctic dry valley lakes where the relatively complete seals of the perennial ice covers result in supersaturated levels of dissolved gases (Wharton *et al.*, 1986, 1987). A similar mechanism is known to result in carbonate precipitation in the Arctic (Hall, 1980).

A search of the recently re-issued Mariner 6/7 Infrared Spectrometer data in the wavelength region between 2 and 6 mm failed to confirm the presence of carbonates. Due to the fact that no spectral footprints appear to directly overlie the layered deposits, and that deposits may be blanketed by an eolian mantle, this negative result is inconclusive. We feel that the canyon deposits are still a prime site for future searches for carbonates on Mars.

The detection of carbonates at these sites may have important implications for the search for evidence for past life on Mars (McKay, 1986).

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CHEMICAL EVOLUTION: A SOLAR SYSTEM PERSPECTIVE

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During the last three decades major advances have been made in our understanding of the formation of carbon compounds in the universe and of the occurrence of processes of chemical evolution in the solar system and beyond. This has been made possible by the development of new astronomical techniques and by the exploration of the solar system by means of properly instrumented spacecraft. We summarize here some of the major findings made as a result of these observations.

1. Interstellar molecules. By means of radioastronomy and microwave spectroscopy it has been found that the interstellar medium contains a diversity of molecules made of biogenic elements (H, C, N, O, S, P, etc.). About 75% of the molecules contain the element carbon, that is to say, they are organic. Formaldehyde was the first organic molecule discovered in interstellar space. The other molecules vary in complexity from diatomic species, such as CO, to molecules with thirteen atoms, such as cyanopentaacetylene. Using other methods of astronomical observation and chemical analysis, much more complex molecules have been found in interstellar space, namely polycyclic aromatic hydrocarbons and other probable precursors of different forms of carbon (amorphous, graphitic, and with diamond-like structures). It appears obvious that the immediate sources of most of the carbon-rich molecules are the carbon-rich stars. There, upon combination of carbon with itself and with other biogenic elements (in the atmospheres of stars, or in their circumstellar shells), the newly formed molecules are ejected into the cold interstellar space. It is only in this way that one can explain the formation of cyanoacetylene and all the higher homologues up to cyanopentaacetylene found in the circumstellar shells of some of these carbon-rich stars. It should be pointed out that one of the more recent discoveries is that of PN, which is the first molecule with the element phosphorus detected in interstellar space. Some of the interstellar molecules are considered be the primeval precursors of the biochemical compounds present in living matter. Indeed, in experiments carried out in terrestrial laboratories, twelve of these molecules (H_2, H_2O, NH_3, CO, CH_2O, CH_2S, CH_3CHO, RCHO, HCN, HC_2CN, H_2NCN, PN, or H_3PO_4) have been demonstrated to yield the basic building blocks of proteins, nucleic acids and membrane lipids. It should also be said that most of the above interstellar molecules are also present in comets.

2. Comets. Comets are without doubt the most interesting bodies in the solar system from the point of view of chemical evolution and the origin of life. They contain large quantities of organic molecules and related chemical species which have been detected in the past by optical and microwave spectroscopy and more recently by mass spectrometry. The spacecrafts sent by the Soviet Union and the European Space Agency to Halley's comet have provided a wealth of information concerning the chemical organic composition of this comet. Aside from the finding of water, ammonia, carbon monoxide, carbon dioxide, hydrogen cyanide, formaldehyde, and presumably a number of heterocyclic (e.g., adenine) and other interesting organic compounds, one of the most surprising observations was the finding of what appears to be the first homologous polymeric molecule present in comets: Polyoxymethylene, or POM, which is a homopolymer of formaldehyde. This supports the concept that comets are aggregates of interstellar molecules condensed prior to the formation of the solar nebula, out of which the solar system was formed. The observation of the pristine nature

of the matter in comets has been held since the beginning of the study of these interesting objects of the solar system. Another observation supporting the pristine nature of comets is the low density determined for Halley's comet, which is indicative of the absence of any significant differentiation of metamorphic processes occurring in such objects.

3. The Jovian planets and Titan. The molecular composition of the atmospheres of the giant planets shows the presence of simple organic compounds in their most hydrogenated forms, such as methane and other hydrocarbons. One could expect this because the large mass of these planets is capable of retaining the hydrogen which is lost in the smaller bodies. However, a close examination of their atmospheres, as well as that of Titan, shows the presence of a number of the same organic molecules which we have encountered first in interstellar space and then in comets. In the case of Jupiter and Saturn, due to the highly dynamic nature of the convection processes occurring in their atmospheres, where large masses of gases and other matter go through regions of different thermal history, one may expect to find concentrations of different organic molecules in conditions of steady state since organic compounds must continuously be formed and destroyed. The thermal processes occurring in the lower layers of the atmosphere of Jupiter would rule out the possibility of chemical evolutionary processes leading to the formation of labile biochemical compounds. Thus it may not be useful to search in these planets for biochemical compounds such as amino acids, or any other important building blocks of biochemical molecules. On the other hand, adenine and other polymers of hydrogen cyanide may be found in the oceans of Titan as a result of the photocondensation of hydrogen cyanide present in its atmosphere. Indeed Titan may turn out to be a storehouse of organic and biochemical compounds preserved in its ocean at very low temperatures.

4. Asteroids and parent bodies of meteorites. Recent observations have indicated that about three-fourths of the asteroids are characterized by having a reflectance spectra somewhat similar to that produced by carbonaceous chondrites. The presumption is made that the composition of the dark asteroids is somewhat similar to that of carbonaceous chondrites. This observation is in line with the widely held hypothesis that the asteroidal belt is probably the major source of the carbonaceous chondrites which have fallen on Earth. As it is known, since 1806 when the first of the carbonaceous chondrites, the Alais meteorite, was analyzed, this group of meteorites contains substantial amounts of water and organic compounds. Among the organic compounds, amino acids and other biochemical molecules have been found. Some of the recent analyses have established the presence of more than 70 amino acids. Among them there are eight amino acids that are usual components of proteins. Some of the others are only found occasionally in biological systems as metabolic products but the remaining amino acids are completely alien to life. It should be clear that in all the cases examined so far (excluding the few cases of possible terrestrial contamination) the amino acids have been found to be racemic mixtures, that is to say, there is an equal amount of the enantiomeric forms D and L for each amino acid. This is the best demonstration that these compounds are indigenous to the meteorite and that they were synthesized by chemical processes, prior to or at about the time of the formation of the solar system. It should also be said that no significant amount of polymers of amino acids, or of other biochemical building blocks, have been isolated from meteorites and unequivocally characterized. This observation is important because it provides an insight into the limitations of the processes of chemical evolution in dark asteroids or the parent bodies of carbonaceous chondrites. Since the Soviet Union is sending probes to Phobos and Mars, 1988-1998, it is appropriate to point out that the characteristic features of Phobos are somewhat comparable to that of the dark asteroids. Therefore it will be interesting to find out if the analyses performed on Phobos demonstrate the presence of amino acids or related biochemical molecules.

5. Terrestrial planets, Earth. The current theory of the origin of the Earth-Moon system suggests that a body the size of Mars collided with the proto-Earth, injected most of the iron into the core of Earth, melted the proto-Earth, and as a result of the impact a significant portion of Earth mantle was ejected into an orbit around Earth, which eventually aggregated and coalesced to form what is the Moon. It is obvious such a catastrophic process would have thrown out of the proto-Earth most of the water and other volatile components. If such was the case one has to ask the source of the secondary primitive atmosphere and hydrosphere after such an event took place. There are two probable sources; additional outgassing from Earth's interior, and capture of planetesimals, comets and other solar system bodies containing these volatiles. We have argued elsewhere that comets could have contributed to primitive Earth as much as 10^{23} grams of matter. Since water and organic compounds are major components of comets this means that primitive Earth captured substantial amounts of simple organic compounds which were probably used as precursors of biochemical molecules. In fact if only 10% of the captured cometary material was involved in this process, this is equivalent to about 10,000 times the total amount of the matter in Earth's biosphere. In an anoxic Earth, whether the compounds are pyrolyzed, or not, upon impact, is irrelevant, because the new molecules formed, e.g., hydrogen cyanide, can recombine with water and ammonia to generate again amino acids, purines and other biochemical molecules.

6. Terrestrial planets, Mars. One could argue that similar collisional processes occurred during the formation of Mars. This was probably the case, but there are several major differences between Mars and Earth. The most significant difference is the fact that the mass of Mars is roughly about one-tenth of that of Earth. This immediately limits the capacity of internal heat generation by Mars and at the same time the ability to retain the volatiles in its atmosphere. A second major difference is that Mars is closer to the asteroidal belt and therefore it could, in principle, receive many more impacts from asteroidal bodies than Earth. A third difference is that Mars is more distant from the Sun than Earth, and that the amount of solar radiation falling on the surface is proportionately less. Thus, it is more difficult for Mars to keep the water liquid although it was probably possible on early Mars, by means of an appropriate greenhouse effect. Indeed, the observations made by the Mariner and Viking spacecrafts point out the presence of extensive "fossil" fluvial features indicating the existence of large bodies of liquid water in the past. The question arises, "Where is the water that was present during the first 800 million years of Mars history?" One of the theories suggests that a substantial portion of this liquid water and atmosphere was lost from the planet by one or several catastrophic collisions with the planet a long time ago. Small impacting cometary bodies will add volatiles, but large asteroidal bodies would deplete the atmosphere of a small planet. This depletion, together with the limited internal heat production was such that it became impossible for the planet to recapture or to generate the water and other volatiles to replenish the ancient Mars hydrosphere and atmosphere. So this leaves us with the question about the possibility of formation of biochemical compounds on the primitive Mars environment during the first 800 million years. An answer to this question can only be obtained if appropriate missions are planned for Mars. From the point of view of exobiology the primary objective should be to examine ancient sediments (subsurface layers of channels, river basins, lakes, and "mud-flower" impact craters) for the presence of organic compounds. The criteria for selecting the different sites to be examined should be studied carefully and follow a systematic approach. Thus, the Mars Observer Mission should be followed within a short time by a Mars Network Mission capable of using appropriately instrumented surface penetrators. This mission, as suggested by several exobiologists, should be planned in accordance with the best judgements and conclusions arrived at from the observations made by the Mariner, Viking and Mars Observer Missions. At a later appropriate time and in light of the findings made by these past four missions, especially the Network Mission, plans should be made for a Mars-Rover

Sample Return Mission. The latter mission should include deep drilling devices and sophisticated analytical instruments, such as combined laser beam-mass spectrometry, for the identification of organic compounds buried in the rocks or deep layer sediments.

7. Europa. Europa is different from the other three major Galilean satellites in that it has a uniformly rounded surface made of a thick layer of solid water ice. The uniqueness of its surface is that it does not show any significant impact craters but on the other hand it shows extensive linear markings several thousand km long and about km wide. These are considered to be massive cracks on the ice crust caused by the tides in its subsurface oceans which result from the gravitational interaction of this satellite with Jupiter. The presence of substantial amounts of water (~ 6%) and its density of about 3 is taken as evidence of a composition somewhat similar to that of C2 carbonaceous chondrites. These chondritic meteorites have a density of about 2.7, contain ~ 2.5% carbon and ~ 13% water, the rest being primarily silicates. Therefore it would not be surprising if this satellite would contain, in addition to water, substantial amounts of organic compounds similar to those present in C2 carbonaceous chondrites. But, perhaps what is more important is that the mutual interaction of the different organic compounds in the presence of fluid water under favorable primordial conditions may have pushed the organic evolutionary processes to a more advanced degree of organization than that observed in meteorites. If this were the case an appropriate landing mission to this satellite may provide a key to the understanding of the intermediate steps in the processes of chemical evolution of carbon compounds in the solar system, which on Earth led to the origin of life. The possibility of survival of terrestrial microorganisms in the subsurface oceans of Europa has been discussed in the recent literature. Indeed, a good terrestrial analog is offered by the microbial ecological community inhabiting the cold subsurface waters of the perennially frozen lakes of Antarctica.

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PHYLOGENETIC PERSPECTIVE AND THE SEARCH FOR LIFE ON EARTH AND ELSEWHERE

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Studies in molecular phylogeny over the past decade have provided a quantitative outline of the evolutionary relationships among known lifeforms. Because of their ubiquity and constancy of function, the ribosomal RNA (rRNA) sequences are particularly useful for molecular phylogenetic analyses. Employing rRNA sequence similarities, C. R. Woese and colleagues have defined the three primary lines of evolutionary descent: the eukaryotes, the eubacteria, and the archaebacteria.[1,2] The rRNA structures of the three primary lineages share many features that are identical or demonstrably homologous, proving that all known, extant organisms are derived from a common ancestor, the progenote. The rates of change of sequences in different lineages, at various times during their evolution, differ significantly. Thus, phylogenetic trees cannot be interpreted in terms of absolute time. It is nonetheless clear that the eukaryote nuclear line of descent arose as early as the two prokaryotic lineages. The major organelles, the chloroplasts and mitochondria, are unequivocally of eubacterial origin. The nature of the progenote in principle may be inferred by the identification of biochemical features that are common to the three primary lineages.

Because terrestrial organisms were derived from a single ancestor, we have no perspective on the potential diversity available to lifeforms that might arise elsewhere. Thus, the discovery of living or fossil organisms on Mars would be of profound importance. Their commonalities with and differences from terrestrial organisms would provide entirely new insight into the origins of life and the fundamental properties of biological systems.

Any search for microbial life on Mars cannot rely upon cultivation of indigenous organisms. Only a minority of even terrestrial organisms that are observed in mixed, naturally-occurring microbial populations can be cultivated in the laboratory. Consequently, we are developing methods for analyzing the phylogenetic affiliations of the constituents of natural microbial populations without the need for their cultivation.[3,4] This is more than an exercise in taxonomy, for the extent of phylogenetic relatedness between unknown and known organisms is some measure of the extent of their biochemical commonalities. In one approach, total DNA is isolated from natural microbial populations and 16S rRNA genes are shotgun cloned for rapid sequence determinations and phylogenetic analyses. A second approach employs oligodeoxynucleotide hybridization probes that bind to phylogenetic group-specific sequences in 16S rRNA.[5] Since each actively growing cell contains about 104 ribosomes, the binding of the diagnostic probes to single cells can be visualized by radioactivity or fluorescence. The application of these methods and the use of *in situ* cultivation techniques is illustrated using submarine hydrothermal vent communities.[6,7]

Regarding planning toward future Mars missions:

1. A specific recommendation that derives from this work is that future intensive searches for life on Mars must employ *in situ* procedures. It is unlikely that Martian microorganisms, if

they exist, will be cultivatable using standard techniques. The capabilities of *in situ* analysis should, as much as possible, focus toward retrieving biochemical information from a single cell. The required technology would be an enormous boon to terrestrial biological studies, as well.

2. Any future consideration of oases for life on Mars must treat not only the surface environment, but also the possibility of subsurface ones. Possible scenarios would include convection-driven aquifers associated with volcanic features.
3. A previously-discussed design concept that was amplified in the discussions should receive further attention. This is the notion of a robust, detector-laden probe, perhaps a cane-like device, that could be used as a prod for inspecting (and sniffing) surfaces and cavities.

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GROWTH OF A MAT-FORMING PHOTOTROPH IN THE PRESENCE OF UV RADIATION

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Knowledge of the survival and growth of microorganisms in the presence of ultraviolet (UV) radiation is important for understanding the potential for life to exist in environments exposed to high fluxes of UV radiation such as early Earth and other planets lacking a UV-absorbing atmosphere. The surface of early Earth was exposed to high levels of RM radiation during the period of the origin and early evolution of life.[1] Fossil evidence suggests that massive microbial mat communities developed in shallow water environments with surface exposure despite these high UV fluxes.[2] It has been suggested that various environmental factors might have protected these early organisms.[3] Results of laboratory tests on the survival of contemporary microorganisms when exposed to short bursts of UV radiation under no-growth conditions have been discouraging.[4]

We have examined the growth of a mat-forming phototrophic prokaryote, *Chloroflexus aurantiacus* in the presence of continuous high UV irradiation under otherwise optimal growth conditions. Our objective was to look for evidence of an intrinsic ability to grow in the presence of UV radiation in a carefully chosen organism known to be unusually resistant to UV radiation,[5] known to be of ancient lineage among the phototrophs,[6] known to resemble ancient microfossils from the Precambrian,[7] and known to be a ubiquitous mat-former. Previously examined microorganisms have not been selected with these criteria in mind and have been poor candidates for models of Precambrian organisms with high intrinsic UV resistance. We chose such a known UV-sensitive organism, *E. coli*, as a control in our model system. Assuming that even a high intrinsic UV resistance would be inadequate for survival and growth in the presence of very high UV fluxes, we selected one environmental factor, iron (Fe^{3+}), as a common, abundant UV-absorbing substance[8] that might protect microorganisms growing in or under iron-bearing sediments. We tested the effectiveness of Fe^{3+} as a UV-protective agent at low concentrations in thin layers.

The organisms were grown in quartz flasks containing organic media. They were grown at their optimal pH and temperature in a simulated anoxic Precambrian atmosphere of 99.5% N_2 and 0.5% CO_2. Continuous visible and near infrared radiation were provided along with a continuous exposure to UV radiation from a germicidal lamp. Some cultures were also incubated under oxic conditions. Iron was mixed in a silica gel poured to various thicknesses and inserted in the UV light path as a screen. Growth was monitored by measuring changes in turbidity.

Both *C. aurantiacus* and *E. coli* grew well in the total absence of UV irradiation in our culture system. *E. coli* did not grow under any of the experimental conditions tested, however. Even at the lowest levels of UV radiation used (0.02 Wm^{-2}) *E. coli* cells died and lysed immediately. *C. aurantiacus*, however, grew well although with lowered growth rate and depressed cell yields under oxic conditions with continuous UV exposures of 0.02 Wm^{-2}. It did not grow well under oxic conditions with 0.1 Wm^{-2} UV irradiation. Under anoxic conditions, however, *C. aurantiacus* grew reproducibly under continuous UV irradiation of 0.5 Wm^{-2}. No growth was obtained at 2.0 Wm^{-2}. Iron was an effective UV absorber. A 1.5 mm gel containing 0.02% Fe^{3+} reduced the intensity of

UV radiation measured at the surface of the culture from 2.0 to 0.5 Wm^{-2}.

We conclude that intrinsic UV resistance in some organisms may account for growth, not just survival, of these organisms when exposed to high UV fluxes under otherwise optimal growth conditions in an anoxic environment. We also conclude that Fe^{3+}-bearing sediments of 1 mm or less in thickness may provide an adequate shield against high UV fluxes permitting the growth of microorganisms just below their surface. The penetration of short wavelength visible radiation was observed to be very poor in natural sands, whereas the penetration of long wavelength visible and NIR radiation was quite good thus potentially permitting the growth of masses of phototrophic microorganisms at depths greater than 1 or 2 mm in such sediments despite high UV fluxes at the surface. As long as growth conditions were met, then the evolution and development of microorganisms would not have been hampered by high UV fluxes impinging upon the surface of iron-bearing sediments.

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VIKING AND MARS ROVER EXOBIOLOGY

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Other than Earth, Mars is the planet generating the greatest interest among those researching and contemplating the origin and distribution of life throughout the universe. The similarity of the early environments of Earth and Mars, and the biological evolution that we know occurred on early Earth provide the motivation to seriously consider the possibility of a primordial Martian biosphere.

In 1975 the Viking project launched two unmanned spacecraft to Mars with the intent of finding evidence of the existence of present or past life on this planet. Three Viking Biology experiments were employed: the Labeled Release experiment, the Gas Exchange experiment, and Pyrolytic Release experiment. Each of these three experiments tested for microbial existence and utilization of a substrate by examining the gases evolved from specific chemical reactions. Although the results of these experiments were inconclusive, they inferred that there are no traces of extant life on Mars. However, the experiments did not specifically look for indications of extinct life. Therefore, most of the exobiologic strategies and experiments we suggest for the Mars Rover Sample Return Mission involve searching for signatures of extinct life. The most significant biological signatures and chemical traces to detect include: isotopic and chemical signatures of metabolic activity, anomalous concentrations of certain metals, trace and microfossils, organically preserved material, carbonates, nitrates and evaporites.

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MARS ROVER SAMPLE RETURN:
A SAMPLE COLLECTION AND ANALYSIS STRATEGY FOR EXOBIOLOGY

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NASA is currently considering a Mars Rover/Sample Return mission for the late 1990's. We have been conducting an interdisciplinary effort to consider how such a Mars mission can be realistically structured to maximize the planetary science return. Our focus has been to concentrate on a particular set of scientific objectives (exobiology), to clarify and prioritize the scientific goals, and to evaluate the instrumentation and analyses required to attain those goals. For reasons described elsewhere it is reasonable to search for biological signatures, both chemical and morphological, of extinct life on Mars. Life as we know it on Earth requires the presence of liquid water, therefore, it is important to explore sites on Mars where standing bodies of water may have once existed. Outcrops of layered deposits within the Valles Marineris appear to be ancient lake beds. Because they are well exposed, relatively shallow core samples would be very informative. The most important biological signatures to detect would be organics, microfossils or larger stromatolite-like structures, although the presence of cherts, carbonates, clays and shales would be significant. In spite of the limitations of current robotics and pattern recognition, and the limitations of rover power, computation, Earth communication bandwidth and time delays, we have developed a partial scenario to implement such a scientific investigation. In our poster, we describe in detail the rover instrumentation and the procedures and decisions and IR spectrometer. We will also describe preliminary results from a collaborative effort with SRI's vision group, which indicate the rover will be able to autonomously detect stratification, and hence will ease the interpretation burden on the scientists and lead to greater scientific productivity during the rover's lifetime.

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ELECTRON SPIN RESONANCE (ESR) DETECTION OF ACTIVE OXYGEN SPECIES AND ORGANIC PHASES IN MARTIAN SOILS

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INTRODUCTION. The presence of active oxygen species (O^-, O_2^- and O_3^-) and other strong oxidants (Fe_2O_3 and Fe_3O_4) has been invoked in interpretations of the Viking biological experiments and a model has also been suggested for Martian surface chemistry [1,2]. The non-biological interpretations of the biological results gain further support as no organic compounds have been detected in the Viking pyrolysis-GCMS experiments at concentrations as low as 10 ppb.[3] This apparent non-detection of organic compounds has been suggested as caused by photocatalytic oxidation processes involving active oxygen species.[1-4] At present, one knows very little about the destructive oxidation mechanisms operating on Mars. Since no organic material has been found in Martian surface soils, it is imperative for exobiology and future Mars missions to search for organic compounds preserved in Martian subsurface samples. The highly active, oxidized and weathered Martian soils as indicated in the Viking results emphasized the need to develop sample selection and *in situ* analysis techniques for the Mars Sample Return Mission.

WHY ESR? Electron Spin Resonance (ESR) measures the absorption of microwaves by a paramagnetic and/or ferromagnetic center in the presence of an external magnetic field. A full interpretation of the observed ESR signals provides a description of the magnetic species on an atomic scale. In many instances, ESR has the advantage of detailed submicroscopic identification of the transient species and/or unstable reaction intermediates in their environments. Since the highly active oxygen species (O^-, O_2^-, O_2^-, O_3^- and R-O-O$^-$) are all paramagnetic in nature, they can be readily detected in native form by the ESR method. In addition, ESR can easily detect organic compounds in the form of free radicals generated in UV and/or γ−ray radiation as reaction intermediates and/or end-products. A lightweight magnet assembly (2.5 kg in weight, and 2.0 × 3.5 × 4.5 inches in dimensions) has been built at JPL which in its present form is quite adequate for the detection of paramagnetic Fe^{3+}, active oxygen species and organic free radicals at X-band frequency (9.2 GHz)[5]. A miniature ESR within the given rover science payload constraints (5–10 kg) and power requirements (5–20 watt) can be developed as an effective, non-destructive sample selection and characterization instrument for the Mars Rover/Sample Return Mission. It is shown in the Viking results that average Martian surface samples contain about 3.7×10^{17} molecules/gram of highly active species (1). Thus, a miniaturized ESR spectrometer should have the sensitivity to examine gram to milligram size samples, and minimum or no sample preparation is required.

ESR DETECTION OF ACTIVE OXYGEN SPECIES. Active oxygen species likely to occur in the Martian surface samples have been detected by ESR in UV-irradiated samples containing MgO. A considerable number of ESR studies have been carried out on active oxygen species formed in thermally activated and/or in γ-ray or UV-irradiated oxides (MgO, CaO, ZnO, Al_2O_3, NaO_2 and TiO_2), zeolites and apatites. The ESR studies have indicated that thermal activation, UV and/or γ-ray irradiation of metal oxides produce paramagnetic defect centers (electron trapping sites), which upon exposure to oxygen results in the formation of active oxygen species such as O^-,

O_2^-, and O_3^-. The active oxygen species thus formed in most metal oxides are found to be highly reactive and thermally stable even up to 200°C (MgO). Numerous examples have been reported on the oxidation and/or decomposition of organic compounds involving active oxygen species in an environment comparable to that observed on Mars.[6,7]

DEGRADATION MECHANISMS. A significant portion of the solar UV flux with photons in the 200 to 300 nm wavelength region readily reaches the Martian surface. The active oxygen species thus formed via UV-irradiation in the presence of metal oxides as catalysts could degrade complex organic into simple organic compounds, and finally into CO_2 and H_2O. The reaction mechanisms of oxidation and decomposition processes of organic materials which could occur in the Martian environments may be simplified as follows:

A. Photocatalytic Oxidation:

$$RH \xrightarrow[\text{Hydrogen Abstraction}]{\text{Active Oxygen Species}} R\cdot \tag{1}$$

$$R\cdot + O_2 \longrightarrow ROO\cdot \tag{2}$$

$$ROO\cdot + RH \longrightarrow ROOH + R\cdot \tag{3}$$

B. Direct Photooxidation:

$$ROOH \xrightarrow{h\nu} RO\cdot + \cdot OH \tag{4}$$

$$RO\cdot + RH \longrightarrow ROH + R\cdot \tag{5}$$

$$\cdot OH + RH \longrightarrow H_2O + R\cdot \tag{6}$$

C. Oxidation/Reduction by Transition Metals:

$$ROOH + Fe^{3+} \longrightarrow ROO\cdot + Fe^{2+} + H^+ \tag{7}$$

$$ROOH + Fe^{2+} \longrightarrow RO\cdot + Fe^{3+} + OH^- \tag{8}$$

where RH is an organic compound containing hydrogen. The organic free radicals (ROO·, RO·, and ·OH) formed as reaction intermediates are the reactive oxidants themselves, whereas the polymeric radicals R· are the precursors to further degradation. These organic free radicals are all paramagnetic, and thus can be detected and identified by ESR. In fact, ESR has been successfully applied to the characterization of photooxidation processes occurring in polymeric systems.[8,9]

ESR DETECTION OF ORGANIC PHASES. We have carried out ESR studies of organic phases in C-1, C-2, and C-3 carbonaceous meteorites. These meteorites are known to contain large amounts of high molecular weight organic compounds and the C-2 chondrites show evidence of extra-terrestrial aqueous alteration as indicated by the presence of carbonates in this group. ESR signals attributable to organic free radicals along with the characteristic ESR signals of calcite are detected in the C-2 group (Mighei, Murchison, Murray, Nogoya and Cold Bokkeveld). Carbonaceous chondrites which contain organic compounds have been shown to give rise to detectable ESR signals of organic free radicals arising from kerogen-like materials present.[10,11]

Recent studies[12–14] have suggested that major objectives of a Mars sample Return Mission should include: (1) Searching for evidence of fossil life forms, because a wetter climate once prevailed and life may have evolved and flourished on Mars in the past; (2) Study of the chemical environment in the subsoil and within rocks, because endolithic life forms can survive on Mars, protected from the harsh ionizing radiation and highly oxidizing chemical environment. Thus, ESR can aid in the search of organic compounds in the form of free radicals preserved in Martian subsoils or in organic fossils such as kerogen-like materials which are difficult to detect by the Viking type pyrolysis-GCMS experiments.

CONCLUSIONS. A miniaturized ESR spectrometer system can be developed for the Mars Rover Sample Return Mission. The instrument can perform the following *in situ* Martian sample analyses:

1. Detection of active oxygen species.
2. Characterization of Martian surface chemistry and photooxidation processes.
3. Searching for organic compounds in the form of free radicals preserved in subsoils, and detection of microfossils associated with Martian carbonate sediments.

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THE METABOLISM OF THE ANTARCTIC CRYTOENDOLITHIC MICROBIOTA

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The carbon metabolism of the cryptoendolithic microbiota in sandstones from the Ross Desert region of Antarctica was studied *in situ* and *in vitro*. Organic and inorganic compounds were metabolized by the microbiota, with bicarbonate being metabolized maximally in the light. There was a linear response of photosynthesis to light up to 200–300 μmole photons $m^{-2}s^{-1}$. The community photosynthetic response to temperature was a minimum at –5° C, two optima at +5 and +15° C and a maximum at +35° C. Photosynthetic metabolism occurred maximally in the presence of liquid water, but could occur in an environment of water vapor. Biomass of the cryptoendolithic microbiota was measured as the amount of lipid phosphate present. The *in situ* biomass ranged from 1.92 to 3.26 g carbon m^{-2} of rock and was 2 orders of magnitude less than epilithic lichen microbiota from Antarctica in a location 7° more north in latitude. With these data, it was possible to calculate primary production and carbon turnover in this simple microbiota. Production values ranged from 0.108 to 4.41 mg carbon $m^{-2}yr^{-1}$, while carbon turnover values ranged from 576 to 23,520 years. These values are the lowest and longest yet recorded for any ecosystem on Earth. If life did evolve on Mars to the level of prokaryotes or primitive eukaryotes, the possibility that the organisms "retreated," to the protection of the inside of the rock so that metabolism could continue during planetary cooling, cannot be overlooked.

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FOSSIL LIFE ON MARS

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Three major problems beset paleontologists searching for morphological evidence of life on early Earth: 1) selecting a prospective site; 2) finding possibly biogenic structures; 3) distinguishing biogenic from abiogenic structures. The same problems arise on Mars. My terrestrial experience suggests that, with the techniques that can be employed remotely, ancient springs, including hot springs, are more prospective than lake deposits.

If, on the other hand, the search is for chemical evidence, the strategy can be very different, and lake deposits are attractive targets. Lakes and springs frequently occur in close proximity, and therefore a strategy that combines the two would seem to maximize the chance of success.

The search for morphological evidence of life on Earth during the Archean and Proterozoic (3.9 Ga–0.57 Ga) has been underway for about a century. Most major discoveries were made in the course of non-paleontological investigations. Each has lead to later systematic and frequently successful searches by paleontologists. There are two reasons why paleontologists have often followed others: we had to learn which rock types preserve remnants of unmineralized organisms and, even in those rock types, fossils usually are rare. Many discoveries have resulted from regional mapping.

For instance, if part of the strategy were to search for stromatolites on Mars, the following observations, although admittedly geocentric, should be considered:

1. The only abundant rock types in which these occur frequently are limestone and dolostone. They also occur in some siliciclastic rocks (sandstones) but they are exceedingly rare. Limestones and dolostones are rare in Archean sedimentary rock sequences.
2. It is rare to find a well-preserved limestone or dolostone with no stromatolites, but it is normal to have to search extensively within any such rock body (and in cherts) before finding possible stromatolites.
3. In most environments where stromatolites occur now (lakes, rivers, marine embayments, open ocean), they occupy only a small fraction of the available area. The reasons for this restriction are very poorly understood and warrant further study.

There is one environment where stromatolites occupy a large fraction of the available area—hot springs. These are associated with volcanism and presumably were abundant on Mars. They are readily recognizable on satellite imagery and aerial photographs because of their more or less circular form within which there is an annular arrangement of sediment types—they are targets in every sense of the word. There are reasons other than ease of recognition and abundance of stromatolites that make springs attractive sites for exploration for life:

1. They are sites of chemical disequilibrium that can be exploited as a source of energy for life;

2. The chemical and thermal gradients associated with springs sort organisms into sharply delineated distinctive and different communities, and so diverse organisms are concentrated into relatively small areas in a predictable and informative fashion;
3. Minerals such as silica and calcium carbonate precipitate from spring waters, so maximizing the chances of preservation of organisms;
4. Chemical sediments in which organisms can be morphologically preserved predominate in spring deposits—clastic sediments are relatively rare, in contrast to the deposits of rivers and lakes.

Once possible stromatolites have been located they must be distinguished from similar but abiogenic deposits. On Earth these are of two main types—splash deposits (stiriolites, that form on shorelines and around geysers) and pedogenic deposits (carbonates, silica and iron and manganese hydroxides that form in soil). Making the distinction can be very difficult or impossible without microscopic and chemical analysis. The following types of observation are required:

1. The distribution and nature of associated sediments (i.e. macroscopic facies relationships).
2. Search for diagnostic mesoscopic features (e.g., evidence of sediment coherence such as is provided by microbial mats—ragged desiccation cracks, overfolded laminae; evidence of possibly abiogenic chemical precipitation *in situ*—fitted pisolites; evidence of movement of microorganisms towards the lightconical laminae with rib-like features).
3. Diagnostic microscopic features (fabrics which indicate the former presence of cells, even if the cells themselves are not preserved). Probably only about 1–10% of stromatolites have such features.
4. Chemical discontinuities such as a carbon isotopic difference between oxidized and reduced mineral species in the stromatolite.
5. In the most favorable cases, preserved microfossils. These are extremely rare in carbonate stromatolites, and occur in perhaps 1–10% of chert stromatolites. Preserved molecules derived from cells (biomarkers) are even more rare.

Even with all of these techniques available on Earth, it is difficult to prove that a stromatolite is biogenic. We generally settle for 90% probability. We could improve on this if we understood more about abiogenic structures that resemble stromatolites, and if we knew more about the processes by which minerals precipitate in and around microorganisms, which might allow us to distinguish biogenic mineral fabrics. We do know, for instance, that stromatolites have a distinctive carbon isotopic composition that is different from that of at least some otherwise comparable structures.

If the site selection strategy were to include spring as well as lake deposits, the chances of finding morphological evidence of life would be significantly enhanced. If in addition it was possible to combine a range of macroscopic, mesoscopic and microscopic observations with isotopic analyses, it would be possible to select probable stromatolites from amongst a larger sample set on the surface of Mars.

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THE ANTARCTIC DRY VALLEY LAKES: RELEVANCE TO MARS

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The similarity of the early environments of Mars and Earth, and the biological evolution which occurred on early Earth, motivates exobiologists to seriously consider the possibility of an early Martian biota. Our research is aimed at identifying environments which could have contained Martian life and areas which may presently contain evidence of this former life. Sediments which are thought to have been deposited in large ice-covered lakes are present on Mars. Such localities have been identified within some of the canyons of the Valles Marineris and more recently in the ancient terrain in the southern hemisphere. We are currently studying perennially ice-covered Antarctic lakes in order to develop quantitative models that relate environmental factors to the nature of the biological community and sediment forming processes. These models will be applied to the Mars paleolakes to establish the scientific rationale for the exobiological study of ancient Martian sediments. One biologically important feature of an ice cover is its capacity to thermally buffer the underlying water from the relatively cold external temperature. The mean annual temperature in the Antarctic dry valleys is −20° C, yet the water in the lake does not cool below 0° C. Consequently, microorganisms are capable of thriving in these thermally stable lakes in spite of the cold external environment. By analogy, it is possible that ice-covered lakes on early Mars provided a relatively warm, liquid water environment for early Martian biota. Another feature of perennially ice-covered lakes is their ability to concentrate atmospheric gases in the water column. For example, we have shown that Lake Hoare, Antarctica, has about 300% more oxygen and 160% more nitrogen than would be in equilibrium with the atmosphere. In Antarctica, both biological and abiological processes contribute to the enhanced gas concentrations. Sedimentation and loss through the ice cover of organic carbon produced through photosynthesis represents a biological source of oxygen. Also, the incoming meltstreams carry air in solution into the lake which is concentrated when the water freezes to the bottom of the ice cover. Both of these processes are effective in controlling the gas concentration in the lake water. These concentration mechanisms may have operated in the ice-covered Martian paleolakes, possibly enhancing the concentrations of biologically important gases (e.g., CO_2, N_2) from the thin Martian atmosphere.

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DETECTION OF MICROBES IN THE SUBSURFACE

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If experience on Earth is somewhat parallel to that of Mars the most likely place to detect evidence for life is in the subsurface sediments that have been protected from the intense ultraviolet irradiation and the peroxide-like oxidants that probably have destroyed the organic carbon molecular fossils that might have existed in the sediments. The experience of expanding the known biosphere on Earth with the detection of an active and diverse microbial community in the deep subsurface sediments has relevance to the search for evidence of life on Mars. A collaborative program, Deep Probe, was organized by F. J. Wobber of the Department of Energy and C. F. Fliermans of the Savannah River Laboratory to recover sediments from the deep subsurface. Extraordinary precautions were utilized to control contamination from surface soils and drilling fluids and a successful program to recover the sediments from as deep as 1,000 feet was achieved. The cores from the sediments were recovered through a paring device which removed the outer surface and immediately transferred them to an anaerobic sterile chamber from which they were distributed to the cooperating universities and national laboratories for analysis. Essentially, a microbiota was recovered from the aquifers that contained between 10^3 to 10^6 organisms/g dry wt. They showed diverse metabolic propensities that were clearly different from organisms from surface soils and sediments. The organisms were primarily bacteria with gram positive cell walls dominating the clay formations. These areas showed the least metabolic activities and microbial biomass. The aquifers showed a more heavily gram negative community with a higher biomass. The aquifers were aerobic and strictly anaerobic; facultatively anaerobic and strictly aerobic bacteria were recovered. The details of the findings for the Deep Probe will be published by the research groups shortly.

The point for MRSR is that the subsurface contained microbial life where it was not expected. Microorganisms were detected by their metabolic activity and growth. However, these techniques depend on the ability to detect and grow each type of microorganism. How does one detect all the organisms present in a subsurface sample? Using ultrasensitive detection techniques for "signature biomarkers" it is possible to both determine the biomass (based on universally distributed biomarkers) and the community structure (based on the detection of biomarkers restricted to subsets of the community). Living cells create unique molecules that have distinctive half lives in soils and subsurface sediments. In the subsurface, it was important to determine if the non-culturable microbiota were viable or potentially viable as contrasted with the accumulation of molecular fossils. Bacteria create urionic acid containing polysaccharide exopolymers that are degraded slowly, but they may persist when cells are no longer viable. The cell walls of bacteria contain monomers such as muramic acid, N-glucuronic acid, ketodeoxyoculonic acid, and the lipipolysaccharide-Lipid A. These unusual components can persist in soils after the death of cells. The cytoplasm contains the DNA, RNA, enzymes, and ATP, all of which can persist after cell death and lysis. The membranes contain lipids, and one class of these lipids, the polar phospholipids, are rapidly degraded on the death of the cells by external and internal phospholipases. The phospholipases form neutral lipids from the polar lipids. Petroleum which is a residue of biological material is clearly lipid, but it contains no phospholipids.

The detection of phospholipids offers a mechanism to determine the presence of viable or potentially viable microbes in subsurface sediments. Phospholipids are formed from fatty acids which, in the microbial world, have a sufficiently diverse structure and asymmetric distribution that they can be used as "signatures" of different groups of microbes. Thus, it is possible to quantitatively define the microbial community structure by the patterns of the polar lipid fatty acids (and ethers if the Archaebacteria are included). The development of high resolution chromatographic separations by capillary gas chromatography, or supercritical fluid chromatography of electron withdrawing derivatives for detection by mass spectrometry of the negative ions has provided 100 attomolar sensitivities with no loss in resolution for fatty acids and lipid amines from environmental samples. The lipids also contain information about the community nutritional status as some specific fatty acids or endogenous lipid storage polymers accumulate during periods of stress. Since the techniques involve the isolation and separation of individual "signature" components it is possible to define specific metabolic activities using ^{13}C or ^{15}N labeled precursors. Recently, it has been shown that the tedious procedures of extraction, fractionation, derivatization, and analysis can possibly be developed into a rapidly automated system. The system is based on the manipulation of supercritical fluid extraction of sedimentary samples with fractionation of the lipids based on the differences in polarity. The lipid samples can be trans-esterified in the gas phase and separated chromatographically prior to analysis by tandem mass spectrometry.

The search for evidence of microbial life in the deep subsurface of Earth has implications for the MRSR program. If suitably protected environments can be found on Mars then the instrumentation to detect biomarkers could be used to examine the molecular details. Finding a lipid in Martian soil would represent possibly the simplest test for extant or extinct life. A device that could do a rapid extraction possibly using the supercritical fluid technology under development now with detection of the carbon content would certainly indicate a sample to be returned.

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AUTONOMOUS EXPLORATION SYSTEM: TECHNIQUES FOR INTERPRETATION OF MULTISPECTRAL DATA

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An on board autonomous exploration system that fuses data from multiple sensors, and makes decisions based on scientific goals is being developed using a series of artificial neural networks. Emphasis is placed on classifying minerals into broad geological categories by analyzing multispectral data from an imaging spectrometer.

Artificial neural network architectures are being investigated for pattern matching and feature detection, information extraction and decision making. Neural nets offer several advantages over traditional techniques. A hardware implementation may be put on a single chip for real time data analysis. Nets are robust against noisy and incomplete data and can be designed to provide both an answer and an estimate of the correctness of that answer. Multiple nets may process data from a number of pixels in parallel, speeding computation time.

As a first step, a stereogrammetry net (developed by Niles Ritter in the Cartographic Applications group at JPL), extracts distance data from two gray scale stereo images. This net works by matching pixels of a similar intensity between the two images, and determining the horizontal offset. Near objects have the greatest offset between corresponding points, far objects the least. Resulting distance planes can be overlaid, and viewed in a compact representation where a given distance is assigned a certain color.

For each distance plane, an edge-finding net identifies edges using vertical and horizontal discrepancies in slope between pixels. Objects are outlined by an edge follower, and spectral reflectance data taken in a limited set of wavelengths to determine whether the outlined region is homogeneous. When a subregion is identified a full spectrum is sampled, then sent to a neural net classifier and a feature detection net. The output is the probable mineral composition of the region, and a list of spectral features such as peaks, valleys, or plateaus, showing the characteristics of energy absorption and reflection.

The classifier net is constructed using a "grandmother cell" architecture: 1) an input layer of spectral data (32 analog values, each for a single wavelength in the range of 2.04 to 2.5 microns), 2) an intermediate processor, and 3) an output value. The processor takes the dot product of the input layer with each of the stored memories (each memory represents a general geological class, e.g., carbonates, oxides). The memory with the highest output value is the closest match to the input spectrum, and the spectrum is assigned to that class.

The feature detector is a three-layer feed-forward network that has been developed to map input spectra to four geological classes (Amphiboles, Clays, Borates, and Carbonates), and will later be expanded to encompass more classes. The input layer receives spectral data as above. The hidden layer is an internal representation of the input consisting of 8 nodes that detect features common to a geological class (e.g., Carbonates have an absorption feature around 2.3 microns).

The feature detector net is a software simulation of a system that will later be put into hardware, where the nodes represent transistors to hold analog voltage levels, and the connections represent resistance values between nodes. There are 44 nodes and 288 connections in the current simulation. The weights of the connections are determined by training the net with preclassified minerals using a backward propagation learning algorithm. This algorithm compares the desired output of the net with the actual output for each of the training set of minerals. Weights are adjusted so as to maximize the log likelihood that the desired output occurs. After training, hidden layer performance is analyzed to determine what features are being detected. Features that are characteristic of a mineral class may be valuable for determining composition of a mineral mixture.

Results from the classifier and feature detector nets will help to determine the relative importance of the region being examined with regard to current scientific goals of the system. This information is fed into a decision-making neural net along with data from other sensors to decide on a plan of activity. A plan may be to examine the region at higher resolution, move closer, employ other sensors, or record an image and transmit it back to Earth.

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16. Abstract

This report consists of abstracts of papers presented in the Exobiology and Future Mars Missions Workshop meeting held in Sunnyvale, California during March 1988. The objectives of the workshop were to consider the scientific questions associated with exobiology on Mars and to determine how these questions could be addressed on future Mars missions. The mission that provided a focus for discussions was the Mars Rover/Sample Return Mission. The international nature of future Mars missions was underscored by the participation in the workshop of two Soviet space scientists.

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